Public Health Goal for
Di(2-Ethylhexyl)Phthalate (DEHP)
in Drinking Water

Prepared by

Pesticide and Environmental Toxicology Section
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

December 1997

LIST OF CONTRIBUTORS

PHG PROJECT MANAGEMENT	REPORT PREPARATION	SUPPORT
Project Officer Anna Fan, Ph.D. ***Chemical Prioritization Report Outline*** Joseph Brown, Ph.D. Coordinator David Morry, Ph.D. Yi Wang, Ph.D. ***Document Development*** Michael DiBartolomeis, Ph.D. Coordinator George Alexeeff, Ph.D. Hanafi Russell, M.S. Yi Wang, Ph.D. ***Public Workshop*** Michael DiBartolomeis, Ph.D. Coordinator Judy Polakoff, M.S. Organizer ***Methodology/Approaches/Review Comments*** Joseph Brown, Ph.D. Robert Howd, Ph.D. Coordinators Lubow Jowa, Ph.D. David Morry, Ph.D. Rajpal Tomar, Ph.D. Yi Wang, Ph.D.	***Authors*** Joseph Brown, Ph.D. John Budroe, Ph.D. Marlissa Campbell, Ph.D. John Faust, Ph.D. Andrew Salmon, D. Phil. ***Primary Reviewer*** John Budroe, Ph.D. ***Secondary Reviewer*** Michael DiBartolomeis, Ph.D. ***Final Reviewers*** Anna Fan, Ph.D. William Vance, Ph.D. ***Editor*** Michael DiBartolomeis, Ph.D.	***Administrative Support*** Edna Hernandez Coordinator Laurie Bliss Sharon Davis Kathy Elliott Vickie Grayson Michelle Johnson Juliet Rafol Genevieve Shafer Tonya Turner ***Library Support*** Mary Ann Mahoney Valerie Walter ***Website Posting*** Robert Brodberg, Ph.D. Laurie Monserrat, M.S. Hanafi Russell, M.S.

We thank the U.S. EPA's Office of Water, Office of Pollution Prevention and Toxic Substances, and National Center for Environmental Assessment for their peer review of the PHG documents, and the comments received from all interested parties.

PREFACE

Drinking Water Public Health Goal of the Office of Environmental Health Hazard Assessment

This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. The PHG describes concentrations of contaminants at which adverse health effects would not be expected to occur, even over a lifetime of exposure. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (amended Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHA) to adopt PHGs for contaminants in drinking water based exclusively on public health considerations. The Act requires OEHHA to adopt PHGs that meet the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which scientific evidence indicates that no known or anticipated adverse effects on health will occur, plus an adequate margin-of-safety.
2. PHGs for carcinogens or other substances which can cause chronic disease shall be based solely on health effects without regard to cost impacts and shall be set at levels which OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider the existence of groups in the population that are more susceptible to adverse effects of the contaminants than a normal healthy adult.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. In cases of scientific ambiguity, OEHHA shall use criteria most protective of public health and shall incorporate uncertainty factors of noncarcinogenic substances for which scientific research indicates a safe dose-response threshold.
7. In cases where scientific evidence demonstrates that a safe dose-response threshold for a contaminant exists, then the PHG should be set at that threshold.
8. The PHG may be set at zero if necessary to satisfy the requirements listed above.
9. OEHHA shall consider exposure to contaminants in media other than drinking water, including food and air and the resulting body burden.
10. PHGs adopted by OEHHA shall be reviewed periodically and revised as necessary based on the availability of new scientific data.

PHGs adopted by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations, drinking water standards adopted by DHS are to consider

economic factors and technical feasibility. For this reason PHGs are only one part of the information used by DHS for establishing drinking water standards. PHGs established by OEHHA exert no regulatory burden and represent only non-mandatory goals. By federal law, MCLs established by DHS must be at least as stringent as the federal MCL if one exists.

PHG documents are developed for technical assistance to DHS, but may also benefit federal, state and local public health officials. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not to be utilized as target levels for the contamination of environmental waters where additional concerns of bioaccumulation in fish and shellfish may pertain. Often environmental water contaminant criteria are more stringent than drinking water PHGs, to account for human exposures to a single chemical in multiple environmental media and from bioconcentration by plants and animals in the food chain.

TABLE OF CONTENTS

SUMMARY

A Public Health Goal (PHG) of 12 ppb is developed for diethylhexyl phthalate (DEHP) in drinking water. DEHP is a phthalic acid ester used primarily as a plasticizing additive in the production of polyvinyl chloride resins. DEHP has a very low degree of acute toxicity, with oral LD_{50} values ranging from 26 to greater than 34 g/kg in a variety of species. Toxicity studies have shown the liver and testes to be the principal target organs. DEHP has also been reported to cause adverse reproductive and developmental effects in laboratory animals. Chronic oral administration of DEHP has been associated with a dose-dependent increase in hepatocellular carcinomas in a National Toxicology Program (NTP) bioassay using B6C3F1 mice and Fischer 344 rats, as well as in industry-sponsored repeat studies conducted with additional doses and biochemical analyses for peroxisome proliferation. Both the International Agency for Research on Cancer (IARC) and the California Department of Health Services (DHS) have found DEHP to demonstrate sufficient evidence for carcinogenicity in experimental animals. Using data from an oncogenicity study in rats, the DEHP drinking water concentration associated with a *de minimis* theoretical lifetime excess cancer risk level of 10^{-6} was calculated to be 0.012 mg/L (12 ppb) based on the linear method and an LED_{10} of 33.4 mg/kg-day (human equivalent). Using the non-linear method with the same LED_{10}, a value of 230 ppb was calculated. Based on reproductive and developmental toxicity, a noncarcinogenic value of 100 ppb can be calculated. The Office of Environmental Health Hazard Assessment (OEHHA) adopts the lowest value of 12 ppb as the PHG for DEHP in drinking water because of the current uncertainty in the potential for DEHP to cause cancer in humans.

INTRODUCTION

This document represents an update of our earlier health risk assessment of DEHP in drinking water which provided part of the technical support for the state's Maximum Contaminant Level (MCL) (DHS, 1988). This document does not include all descriptions and information found in DHS (1988), but focuses on newer information and data or new analyses or interpretations of earlier studies.

In preparing our earlier (1988) health risk assessment of DEHP, the toxicological profile for DEHP (ATSDR, 1987), the "Background Papers for Workshop on DEHP (Di[ethylhexyl]-phthalate) Risk Assessment" (April 1988); and the United States Environmental Protection Agency (U.S. EPA) Carcinogen Assessment Group's evaluation of the potential carcinogenicity of DEHP (U.S. EPA, 1986a) were considered and partially incorporated into the document. In preparing this update current assessments by these and other authoritative bodies (particularly IARC) have been taken into account (ATSDR, 1993; WHO, 1992).

PRODUCTION AND USES

The primary use of DEHP is as a plasticizer in the production of numerous polymers, particularly polyvinyl chloride, which accounts for approximately 95% of its consumption (HSDB, 1997). Production of other polymeric products (e.g., other vinyl resins) and other uses (e.g., an organic pump fluid, in dielectric fluid in electrical capacitors, as a solvent, in photographic film) account for the other 5% of its use.

Estimated annual production in the United States (U.S.) of total dioctyl phthalates of which an estimated 90% was DEHP was 300 million pounds in 1985 to 1990, 340 million pounds in 1977 and 250 million pounds in 1982 (ATSDR, 1993, citing Mannsville Chemical Products Corporation and HSDB). Production facilities were described by ATSDR (1993) in Pennsylvania, New Jersey,

Tennessee and Maryland; no production facilities in California were listed. Imports of six million pounds in 1988, and exports of 10 to 40 million pounds annually in 1980 to 1990 were also noted.

The principal use of DEHP is as a plasticizer in polyvinyl chloride (PVC, "vinyl") plastics. A large number of consumer products, and other items such as building materials and furnishings, medical devices and equipment components are manufactured of flexible PVC, and most of these contain up to 40% DEHP as the plasticizer. At least 95% of DEHP produced is applied to this use. There appear to be many other minor applications, including use as a solvent or inert carrier in inks, pesticides and cosmetics, as a vacuum pump oil, for testing air filtration systems and respirators and as the dielectric fluid in electrical capacitors.

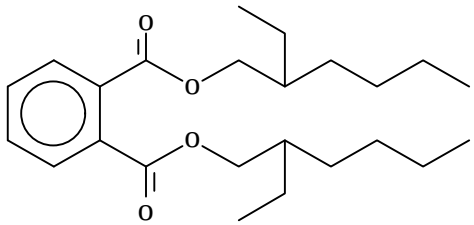
Certain former uses which involved extended food or oral contact (such as plastic food wrap and pacifiers) were discontinued initially because of concerns about the safety of high exposures to DEHP and later due to superior alternatives, although DEHP is still approved for certain uses in food packaging by the U.S. Food and Drug Administration (FDA). The amount of DEHP used in other applications appears to be static or declining for the same reason, with replacement either by other plasticizing agents in PVC plastic, or by different plastics which do not require incorporation of a plasticizing agent to achieve flexibility.

Although no production of DEHP was identified in California, its use in the state appears to be extensive. The Toxic Chemical Release Inventory (TRI) for 1988 (from TRI 1987 to 1994) lists 15 facilities in California where quantities of 0 to 999,000 pounds were stored, handled or incorporated into products. The manufacture and use of items made from DEHP-containing plastic is ubiquitous.

CHEMICAL PROFILE

Chemical Identification

Table 1. Chemical Identification and Molecular Structure

CAS No.	117-81-7
Synonyms	DEHP, bis(2-ethylhexyl)phthalate, BEHP, di-sec-octyl phthalate, dioctyl phthalate, DOP
Molecular Formula	$C_{24}H_{38}O_4$
Molecular Structure	

Physical and Chemical Properties

Table 2. Physical and Chemical Properties

Molecular Weight	390.54 g/mol
Vapor Pressure	1.32 mm Hg at 200°C
Melting Point	-50°C
Boiling Point	230°C at 500 mm Hg
Color/Form	light colored liquid
Odor	slight odor
Specific Gravity	0.9861 at 20°C
Octanol/Water Partition Coefficient	log K_{ow} = 4.89
Solubility	<0.01% in water at 25°C; 0.285 mg/L water at 24°C; miscible with mineral oil and hexane

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURES

Environmental Releases and Transport

DEHP is a widespread contaminant of all environmental media, but the levels present are typically fairly low. Some of the reported measurements of low levels are considered suspect since contamination of laboratory equipment and supplies is often found, leading to false positives or inflated concentration values in low-level environmental samples (ATSDR, 1993). Laboratory results require presentation of adequate background level tests and "blank" readings in order for the sample data to be credible.

Air

DEHP has relatively low volatility at ambient temperatures. Release of the material from plastics containing it into the air are relatively small, but may be important in special circumstances, such as contamination of indoor air. For example, Wams (1987) reported indoor air concentrations of 0.2 to 0.3 mg/m^3 in rooms with newly installed floor coverings. Although only a small proportion of the material is handled, releases into the air from manufacturing facilities may nevertheless involve substantial amounts of material: TRI (1987 to 1994) lists 15 facilities in California where DEHP is handled, each having air emissions in the range 0 to 19,900 pounds. Industrial emissions in the U.S. as a whole were less than 3% of the total annual supply of the compound (ATSDR, 1993). Releases to air have declined in recent years in California and nationwide. Point air emissions in the U.S. declined from 1,040,000 pounds in 1988 to 331,000 pounds, while similar releases in California declined from 149,000 pounds in 1987 to 4,060 pounds in 1994 (TRI 1987 to 1994).

Some air monitoring studies have detected DEHP in ambient air. Levels in remote marine areas average 1 ng/m^3, while average levels in urban and industrial air may be as high as 29 ng/m^3, with much higher levels adjacent to point sources (ATSDR, 1993). Although some airborne DEHP exists as vapor, a substantial proportion is usually associated with the particulate fraction. Airborne DEHP is readily removed to the soil, surface waters and plants, by dry deposition of particles or washout by precipitation. Due to the strong adsorption to soil particles and the

extremely low Henry's Law constant (1.1×10^{-5} atm-m^3/mol), vaporization from contaminated water or soil is not considered a significant source of airborne DEHP.

Water

Water solubility of DEHP is low, but as in the case of airborne material the strong tendency to adsorb to particles results in an additional substantial amount of DEHP bound to suspended sediments in surface fresh water and in marine environments. The total release of DEHP into water is estimated to be similar to that into air, at around 3% of the annual supply. Most of the releases are in industrial effluents and in runoff from waste disposal sites where DEHP-containing industrial wastes and plastics are present. Direct industrial sources appear to be a small part of the total release to water; surface water discharges in the U.S. were 2,800 pounds in 1988 and 960 pounds in 1994. Releases by underground injection totaled 3,100 pounds in 1988, but have been reported as zero since 1992. In California, direct discharges into water appear never to have been substantial. Disposal to public sewage treatment facilities in the state was significant in earlier years (1,500 pounds in 1987) but has declined dramatically, to 256 pounds in 1990: it has not exceeded 20 pounds in any of the years 1991 to 1994 (TRI 1987 to 1994).

DEHP is frequently detected in surface water, ground water and drinking water in the U.S., at levels of up to a few parts per billion (ppb). Of a series of surface water samples, 24% were positive with a median DEHP concentration of 10 ppb; the material was also found in samples from four of the five Great Lakes and several U.S. rivers at levels between 0.5 and 1 ppb. Concentrations in sea water averaged 0.005 to 0.7 ppb (ATSDR, 1993).

DEHP is present in urban runoff; 13% of 86 samples had levels ranging from 7 to 39 ppb (Cole *et al.*, 1984) Levels in leachate from municipal and industrial landfills have been measured varying from < 0.01 to 150 ppm, with geometric mean concentrations in ground water and surface water near hazardous waste sites of 85 ppb and 125 ppb, respectively (ATSDR, 1993). Local concentrations of DEHP in leachates well in excess of its solubility in pure water (0.3 to 0.4 mg/L) have been reported, due to the concurrent presence of common organic solvents such as alcohols and ketones which increase DEHP solubility. This phenomenon also increases DEHP mobility with ground water movement from disposal sites.

Soil

The major route by which DEHP enters the general environment is via disposal of solid wastes. California facilities handling DEHP reported the disposal of 70,078 pounds, and total U.S. industrial disposals amounted to 2,200 pounds in 1988 (TRI 1988). The amount of DEHP reported as going for offsite disposal of solid waste has increased since 1988 in California; figures were 130,546 pounds in 1993 and 126,931 pounds in 1994. The amounts reported in California as sent offsite for recycling have also increased greatly, from 0 in 1990 and previous years to 201,364 pounds in 1994 (TRI 1987 to 1994). These figures are only a small fraction of the total disposal amount, due in particular to the high content of DEHP-containing plastics in municipal waste. ATSDR (1993) estimated that about 2.3 million pounds, 92 % of the annual supply of DEHP, are deposited in landfills annually.

An additional source of DEHP entering soils and sediments is sewage; marine outfalls deposit the material in surrounding sediments, and DEHP-containing sewage sludge is often spread on land.

Since DEHP binds strongly to solid particles, a substantial proportion of material released initially into air or water eventually becomes bound to particles in the soil and sediments. In soil, binding

occurs to both mineral and organic components; its high octanol/water partition coefficient enhances binding to humic acids and other organic material. Williams *et al.* (1995) measured organic carbon normalized sediment/waterpartition coefficients (K-oc) for DEHP which averaged approximately 4.8×10^5, although the value varied with dissolved solids concentrations in the sediment. Background levels of DEHP in soil have not been quantified.

Marine and fresh water sediments have contained average DEHP levels ranging from 6.6 to 1,500 ppb (ATSDR, 1993). High local concentrations are regularly observed near sources of contamination such as incinerators, waste disposal sites and discharge points of contaminated water. In view of the decline in industrial releases of DEHP to air and to sewage treatment works noted above, some of the currently reported contamination of soils and sediments may reflect persistence of DEHP from earlier years when such discharges were substantial. The other major source of input of DEHP to the environment appears to be the extensive disposal of DEHP-containing plastics in municipal wastes.

Accumulation and Degradation

DEHP vapor is predicted to react with hydroxyl radicals in air, resulting in an estimated atmospheric half-life of 12 hours (U.S. EPA, 1987a). The half-life of material adsorbed to particles in air is unknown, but may be longer. In water, chemical hydrolysis occurs too slowly to be of importance, with an estimated half-life of 100 years (Wams, 1987). However, under aerobic conditions DEHP is rapidly biodegradable. It is substantially or entirely degraded in microbial test systems, and the half-life in river water was found to be about one month (Wams, 1987). Degradation also occurs in soil and sediments, although more slowly since adsorption to soil particles reduces the bioavailability. Degradation under anaerobic conditions is slow in sediments, and was not observed in water.

Bioconcentration of DEHP has been observed in invertebrates, fish and terrestrial animals, with reported bioconcentration factors ranging from 54 to 2,700 (ATSDR, 1993). Callahan *et al.* (1979) described the biotic fate of DEHP in some detail. Both single- and multicellular organisms absorb DEHP. Its high octanol/water partition coefficient results in a strong tendency for this compound to partition into the lipids of organisms. The concentration factors tend to be larger for smaller invertebrates, such as crustacea and midge larvae, than for fish.

DEHP is readily metabolized by invertebrates, fish, and other animals, so biomagnification is not observed in typical aquatic or terrestrial ecosystems. DEHP is less biodegradable than short (i.e., less than six carbons) carbon-chain length phthalate esters, more so than several other long-carbon-chain phthalates (i.e., greater than seven carbons), and much more so than persistent compounds such as polychlorinated biphenyls. Several microorganisms, including *Serratia, Penicillium* and *Enterobacter*, have been shown to degrade DEHP.

Uptake of DEHP from soil by plants has been demonstrated. DEHP levels in fish and other aquatic organisms of potential dietary importance may be significant, especially when caught in industrialized areas. Values were found ranging from 2 to 32,000 ppb; one study found DEHP in 33% of 139 samples (not all edible species) with a median concentration of 3,000 ppb (various authors cited by ATSDR, 1993).

Sources of Human Exposure

General Population

It appears that the major source of exposure to DEHP for the general population is via food. Food may be contaminated with DEHP as a result of environmental contamination of source materials. In addition, DEHP may appear in food as a result of transfer from plasticized PVC wrapping materials, containers and processing equipment. This transfer appears to be more extensive with fatty foods. Levels of DEHP in food appear to be generally low (< 1 ppm), although certain processed and/or fatty items may be higher (ATSDR 1993; Perwak *et al.,* 1981). Average daily intakes of 0.25 mg from food, 0.02 mg from water and 0.0004 mg from air were estimated by U.S. EPA (Perwak *et al.,* 1981). Intakes may be lower in more recent years; an average value of 20 µg/day was cited for the population of the United Kingdom (U.K.) in 1986 (WHO, 1992). Uptake of DEHP via skin contact with plasticized PVC materials appears to be minimal, and this has not been considered an important route of exposure for the general population. However regular oral contact with such materials may result in ingestion or transfer of DEHP, as in the case of infants' pacifiers. This use has accordingly been eliminated by voluntary agreement as noted previously.

Occupational Exposure

Workplace exposure may occur for those involved in the manufacture and handling of DEHP: possible routes include inhalation, skin contact and hand-to-mouth transfer. The National Institute for Occupational Safety and Health (NIOSH) estimated that about 340,000 workers were exposed to DEHP (NOES, 1990). Occupational standards for air levels of DEHP have been set by Occupational Safety and Health Administration (OSHA) [Permissible Exposure Levels (PELs) time-weighted average (TWA) = 5 mg/m^3; short-term exposure level (STEL) = 10 mg/m^3] and American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit values (TLVs) the same as the OSHA PELs. Most reported measurements appear to be within the recommended TWA for an eight-hour day. NIOSH recommends that levels should be reduced to the lowest feasible level because of the reported carcinogenicity of DEHP.

Other Highly Exposed Populations

The use of plasticized PVC in a variety of medical devices (e.g., flexible containers for intravenous fluids, transmission tubing, dialysis membranes) results in a possible exposure to patients receiving treatment by means of these devices. These procedures typically result in exposures to DEHP via the intravenous route which are several orders of magnitude larger than those experienced via the oral route by the general population from environmental sources. It appears that exposure situations which involve exposure of blood or similar lipid or protein containing media result in higher uptake of DEHP from PVC equipment than is seen with plain aqueous solutions. As noted above in the environmental context, the solubility of DEHP in pure water is quite low, but is increased by the presence of solvents, lipids or other hydrophobic materials in solution or dispersion. Numerous reports document the extraction of DEHP from blood storage bags, tubing and other plastic materials by blood. Stored human blood was described (Rubin and Ness, 1989) as having typical DEHP levels of 50 to 70 mg/L in 1970, resulting in human exposures of up to 300 mg (5 mg/kg body weight). The contamination with DEHP appears to actually improve the survival of erythrocytes in stored blood, although there are reports of possible deleterious changes in thrombocyte (platelet) function under similar conditions (Fratantoni, 1992).

In addition, the nonionic surfactants typically included in the formulations for injection or intravenous administration of drugs with low aqueous solubility extract DEHP from flexible PVC containers and transmission tubing. Pearson and Trissel (1993) found that polyoxyethylated castor oil and Polysorbate 80 (common formulation ingredients for poorly soluble intravenous drugs) were active in extracting DEHP from plasticized PVC containers, especially in combination with ethanol. After 24 hours in such a container a 25% solution of Polysorbate 80 contained 230 µg/mL DEHP; concentrations of nearly 200 µg/mL were obtained with 5% Polysorbate 80 or polyoxyethylated castor oil and 5% ethanol.

Although most medical procedures involving these devices and materials are limited events experienced at most a few times in an individual lifetime, there are some classes of patient who receive regular treatments for extended periods of time. Examples which have been noted in the literature include patients with various types of hemophilia, who may receive regular transfusions with blood or blood products to supplement the clotting factor which they constitutionally lack, and patients with kidney failure who undergo regular (often weekly) dialysis to remove waste products from their blood.

It has also been found that exchange transfusions in infants, necessary in situations such as Rh blood group incompatibilities, involve extensive exposure to DEHP. In this procedure, the amount of blood used, which has been stored in contact with flexible PVC containers, is proportionately very large (up to three times the infant's normal blood volume). In certain cases the procedure may be repeated several times. Plonait *et al.* (1993) reported that whereas the DEHP levels in infants' blood prior to exchange transfusion and in control infants were usually below the detection limit of 1 µg/mL, and in all cases below 2 µg/mL, after the procedure the median blood serum level of DEHP was 14.5 µg/mL (range 6.1 to 21.6 µg/mL). The plasma concentration of DEHP in transfused blood after passing through the administration set and blood warmer was 44.8 µg/mL (range 4.3 to 153.1 µg/mL). Total exposure to DEHP during the procedure ranged from 1.2 to 22.6 mg/kg body weight, after allowing for removal of some DEHP in waste blood. An earlier study (Sjöberg *et al.*, 1985) reported a median serum concentration of 8.3 µg/mL (range 3.37 to 11.08 µg/mL) after exchange transfusion with blood in which the median plasma concentration of DEHP was 54.6 µg/mL (range 36.82 to 84.94 µg/mL).

METABOLISM AND PHARMACOKINETICS

Absorption

Up to 25% of doses of DEHP administered orally to human volunteers (30 mg DEHP once or 10 mg/day over four days) were excreted in urine (DEHP plus metabolites) indicating some level of absorption from the oral route (Schmid and Schlatter, 1985). The actual level of absorption is likely to be higher because of evidence of significant level of biliary excretion (see below).

DEHP is well absorbed following oral exposure in rats, with at least 55% of an administered oral dose of 2 g/kg absorbed (Rhodes *et al.*, 1986). Because of the presence of the esterase responsible for its metabolism in the small intestine, DEHP may be absorbed intact or its primary metabolites mono-(2-ethylhexyl)phthalate (MEHP) and 2-ethylhexanol may be the primary compounds absorbed. It has been suggested that at low doses, almost all DEHP is converted to MEHP before absorption whereas at high doses, some intact DEHP may be absorbed as well (Albro, 1986; Albro *et al.*, 1982).

DEHP is absorbed by the inhalation route of exposure based upon evidence from both humans and experimental animals. Adult volunteers exposed to 0.5 or 2 mg DEHP/m^3 for five days exhibited accumulation of DEHP in blood and elimination in urine (Kodeikh, 1985). Metabolites of DEHP were detected in urine and significantly increased over the workday among workers in an industry using PVC where inhalation exposure of DEHP was significant (up to 1.2 mg/m^3) (Dirven *et al.*, 1993). Evidence of lung absorption also comes from the appearance of metabolites of DEHP in the blood and lung tissue of infants exposed during respiratory therapy using PVC tubes (Roth *et al.*, 1988). Peroxisome proliferation has also been observed in the livers of pregnant rats exposed to DEHP aerosol during gestation (Merkle *et al.*, 1988).

Several studies have demonstrated that dermal absorption of DEHP is low. Fractions of < 1% to 3% of dermally applied doses of [^{14}C]-DEHP were absorbed and excreted in Guinea pigs after 24 hours exposure (Ng *et al.*, 1992; Chu *et al.*, 1996). After 14 days, the fraction excreted increased to nearly 10% of the applied dose, while the level in the dosed skin decreased from 11% to 0.09% indicating some redistribution from the skin (Chu *et al.*, 1996). The effective dose in the skin (applied dose minus that not absorbed) appeared to be nearly completely absorbed by the seventh day following application. In rats, approximately 95% of a dose of 30 mg/kg was still on the skin after seven days of application (Elsisi *et al.*, 1989). Likewise, an NTP assay indicated that after five days of dosing with a dermally applied dose of 30 mg/kg body weight, only 5% of the dose appeared in urine or feces and 93 to 95% was recovered at the site of application (Melnick *et al.*, 1987). *In vitro* studies with epidermal membranes suggest that human skin is approximately half as permeable to DEHP as rat skin (Scott *et al.*, 1987). Estimates of human dermal absorption rates have ranged from 0.016 to 1.06 mg/cm^2-hour (Scott *et al.*, 1987; Deisinger *et al.*, 1991).

Distribution

Studies of human tissues indicate the presence of DEHP in adipose tissue and kidneys (most likely from oral exposure), although concerns have been raised that inadvertent contamination of samples or tissues prior to analysis may occur (ATSDR, 1993). Evidence from a variety of experimental animals (rat, pig, dog, marmoset) treated orally or dermally with DEHP indicates distribution within four days to a number of sites including liver, adipose tissue and muscle (Ikeda *et al.*, 1980; Elsisi *et al.*, 1989; Melnick *et al.*, 1987; Rhodes *et al.*, 1986). An autoradiographic study using orally administered single doses of ^{14}C-DEHP in mice showed wide distribution to many sites with the exception of the central nervous system (CNS), bones and thymus (Gaunt and Butterworth, 1982). Another study, however, identified the retention of a minimal amount of DEHP in the brains of young mice exposed orally to 0.7 mg DEHP (Eriksson and Darnerud, 1985). Radioactivity from administered radiolabeled DEHP has been shown to cross the placental barrier in both rats and guinea pigs (Singh *et al.*, 1975; Kihlstrom, 1983).

No studies have demonstrated that DEHP bioaccumulates in human or animal tissues, although adipose tissue does appear to retain DEHP or its metabolites (Tanaka *et al.*, 1975).

Metabolism

The bulk of evidence regarding the metabolism of DEHP comes from studies in which the compound is administered either orally or intravenously, two of the more likely routes of human exposure. The primary metabolite of DEHP is the monoester MEHP, which is a product of the lipolytic cleavage of DEHP by esterases/lipases. Evidence from studies showing the same spectrum of metabolites when either DEHP or MEHP are administered orally to rats suggests that the formation of MEHP is the key intermediate in DEHP metabolism (Rock and Viau, 1978). A small amount of phthalic acid may be generated by the cleavage of both side chains from DEHP,

although this reaction is impeded by the inhibition of esterases by the proximity of the carboxyl group formed by the first cleavage (Albro and Lavenhar, 1989). The aliphatic ethylhexyl moiety of the MEHP may undergo ω- or ω-1 oxidation (at one or more sites) to produce a number of products. This moiety also may undergo α- or β-oxidation by alcohol or aldehyde dehydrogenase, which may ultimately reduce the length of the side chain in further oxidative steps. Studies in rat liver and kidney suggest cytochrome P_{450} is responsible for the ω and ω-1 oxidation, with at least two isozymes contributing. No oxidation of DEHP itself is thought to occur and there is no evidence that the aromatic portion of DEHP is degraded (Albro and Lavenhar, 1989).

The other product of the lipolysis of DEHP, 2-ethylhexanol, can undergo β-oxidation producing urinary metabolites which include keto acid derivatives and 2-ethylhexanoic acid (ATSDR, 1993; Albro and Corbett, 1978). DEHP and MEHP also probably stimulate their own metabolism by induction of ω- and peroxisomal β-oxidation (Lhuguenot *et al.*, 1985). The induction of P_{450} by DEHP appears to be relatively specific for isozymes which carry out terminal oxidation. Chronic exposure would thus be expected to result in a higher proportion of metabolites oxidized by this enzyme and this has been confirmed experimentally in rats (Albro and Lavenhar, 1989).

The esterases responsible for the formation of MEHP are distributed widely throughout the tissues, having been identified in the liver, kidney, lungs, skin, plasma, pancreas and the intestinal mucosa. A lipase responsible for the metabolism of DEHP has been isolated from rat pancreas (Albro and Lavenhar, 1989). With the pancreas as a source of lipase, the metabolic conversion of DEHP to MEHP is greatly facilitated in the gastrointestinal tract. It has been proposed that at low level of oral exposure, the enzyme is capable of complete metabolism of DEHP before absorption (Albro, 1986). At high levels of oral exposure, however, there is a question as to whether the metabolic capacity of the gastrointestinal tract becomes saturated, resulting in the absorption of unmetabolized DEHP. Hydroxylated metabolites of DEHP have been identified in the skin of guinea pigs exposed dermally to DEHP (Ng *et al.*, 1992).

Data from human subjects exposed to DEHP intravenously from transfusions and hemodialysis indicate that DEHP is converted to MEHP (Sjöberg *et al.*, 1985; Pollack *et al.*, 1985). While levels of DEHP are higher initially, there is a rapid decline in DEHP ($t_{1/2}$ = 10 hours) with a concomitant rise in MEHP until levels of the two compounds are approximately equal. Blood levels of phthalic acid rise as both DEHP and MEHP disappear from the blood. In rats, a comparison of the conversion of DEHP to MEHP between oral and intraarterial (IA) or intraperitoneal (IP) doses indicates that approximately 80% of the oral dose of DEHP is converted to MEHP whereas only 1% of an ia or ip dose is converted to MEHP (Pollack *et al.*, 1985).

At least eight oxidation metabolites have been identified in the urine of humans exposed orally to DEHP (Schmid and Schlatter, 1985). No single oxidation product constituted the majority of the metabolites, although four metabolites (including MEHP which made up approximately 6 to 12% of the excreted compound) comprised about 90% of the total excreted compound. About 65% of the metabolites were excreted as glucuronide conjugates.

Glucuronic acid conjugation occurs with MEHP and the products of its oxidation. The relative level of glucuronidation across species is rat (none) < mouse, hamster < primates (including humans) (Albro and Lavenhar, 1989). The absence of glucuronidated metabolites in rats does not appear to result from a lack of glucuronyl transferase, but rather higher glucuronidase activity (Albro and Lavenhar, 1989, citing Albro, 1986). A recent study conducted with mice administered ^{14}C-labeled MEHP demonstrated the presence of β-glucose conjugated compounds as a minor conjugated metabolite (~3%), with the linkage forming directly with the phthalic acid moiety by

ester linkage with the glucose (Egestad and Sjöberg, 1992). Conjugation with glutathione, sulfates and amino acids does not appear to occur in rodents (ATSDR, 1993).

Human exposed to DEHP orally produced a qualitatively similar profile of urinary metabolites relative to humans exposed via the intravenous (IV) route (Schmid and Schlatter, 1985; Albro *et al.*, 1982; Albro and Lavenhar, 1989).

Excretion

The primary routes of elimination of DEHP or its metabolites from the body are the urinary and fecal routes, independent of the route of exposure. By the oral route fecal elimination of unabsorbed DEHP may be supplemented by a biliary contribution, thereby confounding estimates of total absorption. Estimates of biliary excretion rates have ranged from 5 to 20% in several experimental animals (Huber *et al.*, 1996). Depending on the dose levels received by the oral route, the fecal component to elimination may be greater because of an increase in the fraction of the compound which remains unabsorbed. A urinary half-life of 12 hours was estimated from data from human volunteers receiving an oral dose of 30 mg DEHP (Schmid and Schlatter, 1985). This value compares favorably with estimates of urinary half-life of approximately 10 to 18 hours determined in experimental animals (Huber *et al.*, 1996). Approximately 5% of a dose of 30 mg/kg applied dermally over seven days was eliminated in the urine (3%) and feces (2%) of rats (Elsisi *et al.*, 1989).

A recent review of the data regarding the elimination of DEHP from humans has suggested that there is considerable human variation and data are lacking regarding the biliary excretion component to human DEHP elimination (Huber *et al.*, 1996). It was noted that the relative contribution of urinary elimination in humans is generally lower than in experimental animals, although there is a question as to whether high dose levels in the animals may have influenced the elimination rates by different routes.

TOXICOLOGY

Acute Toxicity

Acute Toxicity to Aquatic Organisms

An acute LC_{50} value for the fresh water *Daphnia magna* was reported to be 11,100 µg/L (U.S. EPA, 1980b). The LC_{50} values for the midge, scud and bluegill exceeded the concentrations tested at 18,000, 32,000 and 770,000 µg DEHP/L, respectively. Acute fresh water test results, conducted with a diverse group of fish and invertebrate species, were determined for five phthalate esters. The acute LC_{50} values, with one exception, exceeded 1,000 µg/L.

It has been suggested that the earlier published aquatic toxicity data are inadequate (Wams, 1987) since the reported nominal concentrations of DEHP may bear little relation to the true concentrations in solution. This is because 1) a certain portion of the DEHP in water is not bioavailable as a result of biodegradation and adsorption to surfaces and particles, and 2) some reported LC_{50} values considerably exceed the solubility of DEHP in water. The excess DEHP is apparently dispersed in water by adsorption and the formation of colloidal "solutions."

More recent studies have emphasized the relationship between toxicity to aquatic organisms and solubility, and also examined the variation in toxicity with chemical structure (and thus solubility) of various phthalate esters. For example, Adams *et al.* (1995) performed acute aquatic toxicity studies with 14 commercial phthalate esters and representative fresh water and marine species. There was a general trend for the lower-molecular-weight phthalate esters (C-1 to C-4 alkyl chain lengths) to become more toxic with decreasing water solubility for all species tested, but there were only minor differences in species sensitivity to the various phthalate esters. Phthalate esters with C6 or greater alkyl chain lengths (including DEHP) were not acutely toxic at concentrations approaching their respective aqueous solubilities. Insufficient mortality occurred to calculate either LC_{50} or EC_{50} values or acute no-observed-effect-levels (NOELs) for these esters. The lack of toxicity observed for the higher-molecular-weight phthalate esters resulted from their limited water solubility (less than or equal to 1.1 mg/L).

Chronic toxicity studies were also performed by Rhodes *et al.*, 1995 with commercial phthalate esters and *Daphnia magna* and rainbow trout (*Oncorhynchus mykiss*). For lower-molecular-weight (methyl, ethyl and butyl) phthalate esters, toxicity for both species increased as water solubility decreased. The geometric mean maximum acceptable toxicant concentration (GM-MATC) for *D. magna* ranged from 0.63 to 34.8 mg/L. For higher-molecular-weight phthalate esters, including DEHP, the GM-MATC values ranged from 0.042 to 0.15 mg/L. Survival was equally sensitive and sometimes more sensitive than reproduction. The observed toxicity to daphnids with most of the higher-molecular-weight phthalate esters appeared to be due to surface entrapment or a mode of toxicity that is not due to exposure to dissolved aqueous-phase chemical.

Acute Mammalian Toxicity

Lethality Studies

Data on the acute lethality of DEHP are presented in Table 1. Lethality data are not available from inhalation studies: in two rat experiments, exposure to DEHP for one hour at 23,670 mg/m^3 and for six hours at 600 mg/m^3 did not result in mortality. Acute oral studies with a variety of animals report LD_{50} values for DEHP ranging from 26,000 to 4,000 mg/kg, indicating a low order of acute oral toxicity. In rats, acute oral doses produced marked cloudy swelling of the liver and moderate swelling of the kidney accompanied by granular secretion in the tubules (Shaffer *et al.*, 1945).

These changes indicated that liver and kidney injury contributed to the fatal outcome. The high LD_{50}s obtained from ip and iv administration also point to the low acute toxicity of DEHP. In a study by Lawrence *et al*. (1975), the lethal effect of this compound appeared to be cumulative, since the chronic LD_{50} value for ip administration to mice five times weekly for 10 weeks was 1.36 g/kg, in comparison to a single-dose value of 37.8 g/kg. Autian (1982) concluded that this is because metabolism is required before DEHP produces toxic effects. Shaffer *et al*. (1945), using a minor modification of the cuff test, applied single doses of DEHP to intact rabbit skin for 24 hours and observed the animals for 14 days. A dose of 19,722 mg/kg killed two of six rabbits. The authors concluded that the LD_{50} by skin absorption in rabbits was approximately 24,650 mg/kg. No injury to rabbit skin resulted from direct dermal contact with concentrated DEHP.

Acute Dermal Toxicity In Vitro

An absorption study *in vitro* using human and rat epidermal membranes reported irreversible alteration in skin barrier function following exposure to neat DEHP (Scott *et al.*, 1987). Following

contact with DEHP for up to 72 hours, a slight increase in the permeability of human skin was detected. This effect was not significantly different from that caused by hydration (water). DEHP contact with rat skin caused an increased alteration in barrier function relative to human skin despite a shorter contact period (up to 53 hours). The authors stated that this type of alteration might enhance absorption of the chemical in rats, and would be likely to occur *in vivo*.

Acute Ocular Toxicity

Application of 0.5 mL undiluted DEHP in the eye of the rabbit produced no necrosis or damage to the cornea detectable by fluorescein staining, and only transient congestion of the lids (Shaffer *et al*., 1945).

Table 1. Acute lethality data on DEHP (ATSDR, 1987)

Administration Route	**Species**	**LD_{50} (mg/kg)**	**LC_{50} (mg/m^3)**	**References**
Inhalation (1 hour)[a]	Rat		> 23,670 (1457 ppm)	WARF Institute (1976)
Inhalation (6 hours)[a]	Rat		> 600 (37 ppm)	
Oral	Rat	26,000		Patty (1967)
	Rat (Wistar, male)	> 34,000		Hodge (1943)
	Rat (Wistar, male)	30,600		Shaffer *et al.* (1945)
	Mouse	26,000		Patty (1967)
	Mouse	33,500		Krauskopf (1973)
	Guinea pig	26,300		Krauskopf (1973)
	Rabbit	33,900		Shaffer *et al.* (1945)
Intraperitoneal (IP)	Rat	49,000		Singh *et al.* (1972)
	Rat (Wistar, male)	30,600		Shaffer *et al.* (1945)
	Mouse	4,200		Calley *et al.* (1966)
	Mouse (ICR, male)	38,000		Lawrence *et al.* (1975)
Intravenous (IV)	Mouse	1,060		Peterson *et al.* (1974)
IV (sonicated in rat serum)	Rat	2,080		Petersen *et al.* (1974)
Dermal	Guinea pig	10,000		Thomas *et al.* (1984)
	Rabbit	20,000		Thomas *et al.* (1984)
	Rabbit	24,650		Shaffer *et al.* (1945)

[a] Duration of exposure

Subchronic Toxicity and Other Acute Studies

The principal toxic effects of DEHP noted experimentally in mammals involve at first damage to the liver and in some cases the kidneys, and secondly effects on reproduction and development,

notably the production of testicular atrophy and a number of adverse developmental effects. These effects have been studied by a number of investigators and various attempts have been made to relate the observed effects to postulated mechanisms of action of DEHP or its metabolites. These studies, and those chronic or lifetime exposure studies in which possible carcinogenic effects were examined, are described in other sections of this report. Other toxicity studies, including general screening studies and investigation of other possible effects not clearly related to these main classes of response are presented here.

An intermediate-duration study of inhaled DEHP aerosols in rats identified a lowest-observed-adverse-effect-level (LOAEL) of 1,000 mg/m^3 (62.6 ppm) for increased liver weights (males and females), lung weights (males) and foam cell proliferation (males) after four weeks of exposure (Klimisch *et al.*, 1991; 1992). Animals were exposed (head and nose) to respirable particle size aerosol concentrations of 0, 10, 50 or 1,000 mg/m^3 DEHP (mass median aerodynamic diameter < 1.2 µm) for six hours/day for four weeks. All these treatment related effects appeared to reverse after a eight week post-exposure period. Additionally, this study included a fertility assessment (in which no substantial effects on mating performance were noted), details of which appear in the section on developmental and reproductive toxicity.

Brain *et al.* (1996) studied the acute pulmonary toxicity of materials suggested as suitable aerosols for human studies. Materials, including DEHP, suspended in saline (2.5%, w/v) were instilled into the lungs of hamsters. The dose was 3.75 mg/100 g body weight (BW). Control hamsters were instilled with physiological saline. One day later the hamsters were killed and the lungs were lavaged. Biochemical and cellular components of the lavage fluid were then analyzed for indicators of inflammation, edema, bleeding, macrophage phagocytosis, cell injury and cell secretion. Measurements were either within or below control levels. The authors concluded that DEHS, DEHP, corn oil, mineral oil and hexaethylene glycol cause negligible acute pulmonary toxicity in hamsters when given at doses as high as 3.75 mg/100 g BW, but suggested that the chronic effects and pharmacokinetics of these substances warrant further study, particularly if they are used in humans at higher concentrations or for chronic exposures.

A subacute study (Jäckh *et al.*, 1984; Rhodes *et al.*, 1986) compared the toxicity of DEHP in rats and marmoset monkeys. Oral doses of 2000 mg/kg body weight/day were administered to both species for 14 days. Although there was no mortality in either species the rats at this dose level displayed marked toxicity, evidenced by clinical signs (salivation, dehydration), liver enlargement, testicular atrophy and various biochemical indices of damage in the liver. No substantial effects of any kind were noted in the marmosets. It was suggested that this was due both to lower bioavailability of oral DEHP in the marmoset and lesser responsiveness of the primate species to DEHP toxicity. The latter consideration was supported by a study in which marmosets received intraperitoneal doses of 1000 mg/kg body weight/day for 14 days. No morphological or biochemical changes were observed in either the liver or the testes.

Chronic Toxicity

The primary target organs of DEHP have been shown to be the liver and testes. The testicular effects produced by DEHP are discussed in the section on reproductive toxicity.
Groups of male and female Wistar albino rats were fed levels of 50, 200 or 1,000 mg/kg-day DEHP in the diet (Mitchell *et al.*, 1985). Four rats from each experimental group and six control animals were sacrificed on days 3, 7, 14, 28 and at 9 months. Soon after treatment there were morphologic alterations in the bile canaliculi of male rats in the high-dose group. Liver cells exhibited a burst of mitosis after DEHP treatment, with the increased activity greatest at three days in rats treated with 1,000 mg/kg-day. Induction of peroxisomal enzymes (cyanide-insensitive

palmitoyl-CoA oxidase, alpha-glycerophosphate dehydrogenase and catalase), the P_{450} isoenzyme, and an accumulation of fat in the liver was seen throughout the study. More slowly occurring changes were centrilobular loss of glycogen, a fall in glucose-6-phosphatase activity and hypertrophy of hepatocytes. Rats treated with DEHP for nine months with 200 or 1,000 mg/kg-day showed an accumulation of lipid-loaded lysosomes. In general, hepatic changes in females were less pronounced than those observed in males treated with an equal dose.

In a chronic study (Carpenter *et al*., 1953), groups of 24 guinea pigs of each sex were administered diets of 19 or 64 mg/kg-day DEHP for a period of one year. No treatment-related effects were found on mortality, body weight, kidney weight or gross pathology and histopathology of kidney, liver, lung, spleen or testes. A statistically significant increase in relative liver weight was reported in both groups of treated females.

Abnormal liver histopathology was observed in rhesus monkeys receiving transfusions of plasma containing concentrations ranging from 7 to 33 mg DEHP over a one-year period (Jacobson *et al*., 1977). Changes included vacuolated Kupffer cells, foci of parenchymal necrosis, chronic inflammatory cell infiltrates and prominence or hyperplasia of Kupffer cells. Detectable concentrations of DEHP were present in the liver up to five months following cessation of the transfusions. Decreased sulfobromophthalein clearance accompanied the abnormal liver histopathology, indicating DEHP hepatotoxicity in monkeys.

In a recent study (Ganning *et al*., 1987), DEHP was administered to male rats in the diet at concentrations of 0.02, 0.2 and 2.0% for up to 102 weeks. During the entire exposure period a number of key liver enzymes were monitored. Both cyanide-insensitive palmitoyl CoA dehydrogenase and carnitine acetyltransferase were rapidly induced by 2% DEHP; at the two lower doses both enzymes were induced much more slowly, but showed a continuous increase over the two years. Only the high-dose group exhibited an initial induction of cytochrome P_{450} microsomes; after 24 weeks levels decreased, but remained higher than control values. Increased levels of the membrane lipid dolichol occurred in rats treated with two percent DEHP after five weeks; the levels of the phosphorylated intermediate (dolichyl-P) decreased in microsomes as a result of DEHP treatment. The rate of protein glycosylation in liver microsomes was considerably decreased after six weeks. DEHP appeared to interfere with protein turnover, where the half-life of total mitochondrial and microsomal protein was significantly increased.

Developmental and Reproductive Toxicity

Developmental Toxicity

A summary of the results of selected developmental toxicity studies is presented in Table 2. Developmental toxicity has been observed following oral exposure of pregnant mice or rats to DEHP (NTP, 1986; 1988; Tyl *et al*., 1988; Shiota *et al*, 1980; Shiota and Mima 1985). Mice and rats have also been adversely affected by prenatal exposure to DEHP via the ip injection route (Shiota and Mima, 1985; Singh *et al*., 1972; 1975). No clear adverse effects on either dams or offspring were identified in a study conducted by the inhalation route of exposure in rats (Merkle *et al*., 1988).

Gastrointestinal conversion of DEHP to its primary metabolite, MEHP, has been considered as a possible critical factor in the developmental toxicity of DEHP (Shiota and Mima, 1985; Tomita *et al*., 1982; Yagi *et al*., 1980). When given by the injection route, bypassing the gastrointestinal tract, DEHP is a less effective developmental toxicant than when it is given by the oral route.

Conversely, MEHP given by the oral route appears to be a more potent developmental toxicant than DEHP.

Overall, rats appear to be less sensitive to the developmental toxicity of DEHP than are mice. No-observed-adverse-effect-levels (NOAELs) for developmental toxicity in rats following DEHP exposure by the oral route were in the vicinity of 150 to 350 mg/kg-day (NTP, 1986; Tyl, 1988), as opposed to less than 50 mg/kg-day in mice (NTP, 1988; Tyl 1988). Corresponding LOAELs were in the range of 300 to 700 mg/kg-day for rats, and 90 to 95 mg/kg-day for mice. Adverse effects in both species have included reduced viability and reduced fetal or birth weights. Malformations have been observed in mice, but have not been described for rats exposed by the oral route.

Mice

Timed-pregnant CD-1 mice were given DEHP in the diet on gestation days 0 to 17 (Tyl *et al.*, 1988). Dietary concentrations of 0, 0.025, 0.05, 0.10 and 0.15% were determined to have provided doses of approximately 0, 44, 91, 191 or 292 mg/kg-day, respectively. At the two highest DEHP concentrations, maternal body weight was reduced on several gestation days, and relative liver weight was increased. Clinical symptoms of toxicity were limited to lethargy and rough coat, but occurred in all dose groups. Various measures of fetal viability showed significant adverse effects of DEHP given at concentrations of 0.10 or 0.15% in the diet. Fetal body weights were significantly decreased at 0.15% DEHP, and the body weights of female fetuses were also significantly decreased at 0.10% DEHP. The frequency of malformed fetuses, either on a per litter basis or as a percentage of total fetuses, was significantly increased at 0.05, 0.10 and 0.15% DEHP. The malformations observed included external, visceral and skeletal defects. Based on the finding of increased malformations at concentrations as low as 0.05% DEHP in the diet, an LOAEL of 0.05%, or 91 mg/kg-day was determined. The corresponding NOAEL for DEHP was determined to be 0.025%, or 44 mg/kg-day.

In a study of the long-term effects of prenatal exposure (NTP, 1988), DEHP was administered in the feed to timed-pregnant CD-1 mice (P_0 generation). Treatment was restricted to gestation days 0 to 17. Concentrations of DEHP in the diet were 0, 0.01, 0.025 or 0.05%, resulting in average doses of 0, 19, 48 and 95 mg/kg-day. Offspring were delivered and reared normally, with no further treatment. Evaluations were made during the postnatal period for viability, growth and attainment of developmental landmarks. Upon reaching sexual maturity the offspring (F_1) were themselves studied for reproductive potential.

Evidence for maternal toxicity in the P_0 generation was limited to trends for decreasing body weight on postnatal days four and seven. Prenatal mortality was significantly increased at the high dose (95 mg/kg-day), with a corresponding decrease in litter size on postnatal day one. In this same dose-group, there was an increase in the frequency of pup deaths occurring between postnatal days one and four. During the mating trials of F_1 animals, there were minor, transient reductions in the body weights of females. Otherwise, subsequent to postnatal day four, there were no observed adverse effects on growth, viability, development or reproductive performance of F_1 animals. Based on the reductions in viability of the F_1 animals on postnatal days one and four, an LOAEL of 95 mg/kg-day can be determined for developmental toxicity, with an NOAEL of 48 mg/kg-day.

Table 2. Developmental Endpoints for DEHP

Strain & Species	LOAEL	Observation	NOAEL	Reference
CD-1 Mice	91 mg/kg-day	malformations	44 mg/kg-day	Tyl *et al.* (1988)
CD-1 Mice	95 mg/kg-day	reduced viability of F_1	48 mg/kg-day	NTP (1988)
ICR-JCL Mice	190 mg/kg-day	reduced fetal viability	70 mg/kg-day (confidence is limited by low number of litters per dose group)	Shiota *et al.* (1980)
ICR-JCL Mice	IP: 8,000 mg/kg oral: < 250 mg/kg	oral: malformations IP: reduced viability	oral: ND IP 4,000 mg/kg	Shiota and Mima (1985)
Fischer 344 Rats	666 mg/kg-day	reduced fetal body weight	357 mg/kg-day	Tyl *et al.* (1988)
Fischer 344 Rats	313 mg/kg-day	increased post-implantation mortality, decreased avg. litter size, avg. pup wt./litter	164 mg/kg-day	NTP (1986)
Sprague-Dawley Rats	< 4,930 mg/kg x3 (IP)	increased resorptions: decreased fetal weight	ND	Singh *et al.* (1972; 1975)
Wistar Rats	> 0.3 mg/L air, 6 hours/day on gestation days 6 to 15	no clear effects	ND	Merkle *et al.* (1988)

ND - not determined

In another dietary study, ICR-JCL mice were provided with mean daily intakes of 70, 190, 400, 830 or 2,200 mg/kg DEHP throughout gestation (Shiota *et al*., 1980). Decreased maternal weight gain and increased resorption rates were observed in animals given 400 mg/kg or more. A dose of 190 mg/kg-day can be considered the maternal NOAEL for this study. All implants were resorbed at the 830 and 2,200 mg/kg dose levels, and adverse effects on fetal viability were also observed at doses of 400 and 190 mg/kg-day. Reduced fetal weights as well as an increase in the frequency of fetuses having malformations were observed following prenatal exposure to a dose of 400 mg/kg-day. Predominant malformations included neural tube defects (exencephaly and spina bifida). While an NOAEL for developmental toxicity of 70 mg/kg-day can be derived from these data, confidence is limited by the low number of litters (7 to 12) per dose group.

Shiota and Mima (1985) compared the effects of DEHP administered by the oral and ip routes. Groups of pregnant mice were given 250, 500, 1,000 or 2,000 mg/kg orally, or 500, 1,000, 2,000,

4,000 or 8,000 mg/kg by injection on gestation days seven, eight and nine. Resorptions and malformations were significantly increased following oral dosing with 1,000 or 2,000 mg/kg. Anterior neural tube defects (anencephaly and exencephaly) were the most common abnormalities. Indications of increased malformation frequency at the lower doses of 500 and 250 mg/kg complicate determination of a developmental NOAEL from these data. The maternal NOAEL by the oral route was determined to be 1,000 mg/kg. When given by injection, the LOAEL for developmental or maternal toxicity was 8,000 mg/kg, with corresponding NOAELs of 4,000 mg/kg. At this dose level, viability of both maternal animals and fetuses was reduced, but no malformations were observed. Based on the differences in effects between the two routes of administration, the authors concluded that biotransformation in the gastrointestinal tract may be required for DEHP to exert its developmentally toxic effects.

The relative teratogenic potentials of DEHP and its active metabolite MEHP have been evaluated (Yagi *et al*., 1980; Tomita *et al*., 1982). In both of these studies, DEHP and MEHP had similar adverse effects, but MEHP appeared to be the more potent developmental toxicant. Twelve hours following administration of a large oral dose of DEHP to pregnant mice on gestation day eight, both DEHP and MEHP were detected in fetal tissues (Tomita *et al*., 1982). MEHP was presumed to have formed in the dam, and then crossed the placenta, as fetuses younger than gestation day nine are not known to be able to convert DEHP to MEHP. On the basis of these data, it seems possible that MEHP could be responsible for the developmental effects observed following treatment with DEHP.

Rats

Timed-pregnant Fischer 344 rats were fed diets containing DEHP at concentrations of 0, 0.5, 1.0, 1.5 or 2.0% (Tyl *et al*., 1988). Diets with DEHP were provided on gestation days 0 to 20, and resulted in daily doses of approximately 0, 357, 666, 856 or 1,055 mg/kg. Dose-dependent reductions in maternal weight gain reached statistical significance in the three highest dose groups. Maternal food consumption was significantly decreased, and liver weights significantly increased, in all treated groups. Fetal viability was significantly reduced at the highest dose level, and fetal body weights were significantly reduced at DEHP levels of 666, 856 or 1,055 mg/kg. No clear treatment-dependent relationship between DEHP and malformation frequency was identified. Based on the decreases in maternal weight-gain and fetal body weight, the NOAEL for both maternal and developmental toxicity is 0.5% or 357 mg/kg-day.

Using a similar protocol to the NTP (1988) study conducted in CD-1 mice, NTP (1986) administered DEHP in the feed of time-mated, pregnant Fischer 344 rats. P_0 animals were given treated feed from gestation day 0 to 20. On a percentage basis in the feed, DEHP was present at 0, 0.25, 0.50 or 1.0%. These levels corresponded to approximate doses of 0, 164, 313 and 573 mg/kg-day. Maternal weight gain was significantly reduced in the high-dose group, and food consumption was reduced in the high and mid-dose groups. There were no differences between groups in the percentages of fertile matings, the number of implantation sites per dam, or in the frequency of live litters on postnatal days one or four. Post-implantation mortality was increased in both the mid- and high-dose groups, but the effect reached statistical significance only at the mid-dose. Average litter size and average pup weight per litter were decreased in a dose-dependent manner, but the effect on pup weight was statistically significant only at the high dose. Surviving F_1 offspring showed no subsequent adverse effects upon viability, growth, acquisition of developmental landmarks, or on their ability to reproduce upon reaching sexual maturity. Based upon post-implantation mortality, the LOAEL for developmental toxicity in this study was 313 mg/kg-day, with an NOAEL of 164 mg/kg-day.

Singh *et al*. (1972; 1975) demonstrated that DEHP and its metabolites cross the placenta in rats, and can cause embryolethal and teratogenic effects in these animals. Sprague-Dawley rats were given ip injections of 4,930 or 19,860 mg/kg DEHP on days 5, 10 and 15 of gestation. An increased frequency of resorptions and decreased fetal weights occurred in both treated groups. Gross abnormalities were seen only at the high dose of DEHP; malformations consisted of twisted hind legs in one fetus, and hemangiomas of the limbs in 9 out of 41 fetuses.

Pregnant Wistar rats were exposed, in a nose-only inhalation apparatus, to aerosols of DEHP at concentrations of 0, 0.1, 0.05 or 0.3 mg/L air (Merkle *et al*., 1988). Exposure occurred for six hours/day, on each of gestation days 6 to 15. In a pilot study, conducted to determine appropriate concentration levels for the apparatus used, peroxisome proliferation was found to increase in severity with concentrations of DEHP ranging from 0.2 to 1.0 mg/L air. In the main study, peroxisome proliferation was not evaluated, and no other evidence of maternal toxicity was identified at any concentration. Twenty litters in each concentration group were examined using standard teratological techniques. An additional five litters from each group were allowed to come to term. These offspring were raised until postnatal day 21, and evaluated for postnatal developmental endpoints. There were no apparent treatment-related effects on fetal viability or fetal weights, or on any of the postnatal parameters evaluated. Increases in the frequency of hydroureter and dilated renal pelvis were found in the highest concentration group, but the study authors did not consider these changes to have been the result of DEHP treatment.

Rabbits

MEHP injected into previously artificially inseminated female rabbits at critical intervals during gestation caused no significant teratogenic effects (Thomas *et al*., 1979; 1980). Administration of MEHP did not affect the size of the fetus, as evidenced by crown-rump and transumbilical measurements.

Reproductive Toxicity

Breeding Studies

The reproductive toxicity of DEHP was investigated in NTP's continuous breeding test battery (NTP, 1982). Breeding pairs of CD-1 mice were given DEHP in their feed, at concentrations of 0, 0.01, 0.1 or 0.3%. The animals were exposed during a seven-day pre-mating period, a 98-day co-habitation period and a 21-day segregation period. Under this protocol, fertility was completely suppressed in the high-concentration group, and significantly reduced in the mid-concentration group. In a further experiment, cross-over mating trials were performed, to determine if the affected sex could be identified. Regardless of which sex was treated, dietary exposure to the high concentration of DEHP resulted in significant reductions in fertility, demonstrating that DEHP is both a male and a female reproductive toxicant.

As part of the NTP continuous-breeding study (NTP, 1982), animals from the 0.3% DEHP group were compared with untreated controls for sperm parameters, histopathology and hormone levels. In males of the treated group, the percentage of motile sperm and sperm concentration were significantly reduced, the percentage of abnormal sperm was significantly increased and testicular, epididymal and prostatic weights were decreased. The seminiferous tubules of the treated males were severely damaged. Testosterone levels were reduced, and FSH and LH levels elevated, but the differences were not statistically significant. The reproductive tracts of treated females (ovaries, oviducts, uterus and vagina) were significantly reduced in weight in comparison to

controls. This difference, however, was thought likely to have reflected the normal smaller size of the nulliparous female reproductive tract; control animals having experienced pregnancy, while the treated animals had not.

Based upon the significant reduction in fertility of treated pairs, the LOAEL for male and female reproductive toxicity in this study was a dietary concentration of 0.1% DEHP. The corresponding NOAEL was 0.01% DEHP. The study authors did not present their treatment regimen in units of DEHP per kg body weight of the study animals. While body weight and feed consumption data were collected, and mean values can be determined, requirements of the continuous-breeding protocol introduce substantial variation into these parameters. As pointed out by Lamb *et al*. (1987), ". . . since the male and female mice are housed as breeding pairs and the two sexes have such different body weights, it is not possible to determine accurately the relative dose of chemicals on a milligram per kilogram body weight basis. Such data can only be collected in either the range finding study or the separation period at the end of the 14-week mating trial." Additionally, body weight and feed consumption of female mice can be expected to change considerably over the course of gestation.

With the above caveats in mind, average feed consumption and body weight data for the NTP continuous-breeding study on DEHP (NTP, 1982) were presented by Lamb *et al*. (1987): "Analysis of feed consumption showed that the mice consumed between 4.8 and 5.4 g of food per day, regardless of treatment group. Mean body weights for male mice were 36.3 and 34.6 g for control and high-dose groups, respectively, at Week 1 and 36.9 and 37.4 g for control and high-dose groups, respectively, at Week 13." Therefore, an average feed consumption value of 5.1 g/day and an average body weight of 36 g can be used as a reasonable approximation in calculating dose-rates for this study. On this basis, DEHP concentrations in feed of 0, 0.01, 0.1 and 0.3% are roughly equivalent to doses of 0, 14, 141 and 425 mg/kg-day. The LOAEL and NOAEL for adverse effects on fertility of male and female mice are then 141 and 14 mg/kg-day, respectively.

Interactions with other chemicals

The non-additive developmental toxicity of mixtures of trichloroethylene (TCE), di(2-ethylhexyl) phthalate (DEHP), and heptachlor has been studied in 5 x 5 x 5 designs in Fischer-344 rats. Dose levels of 0, 10.1, 32, 101, 320 mg/kg-d for TCE, 0,24.7, 78, 247, 780 mg/kg-d for DEHP, and 0, 0.25, 0.8, 2.5, 8 mg/kg-d for heptachlor were administered by gavage on days 6-15 of gestation. The dams were allowed to deliver and the pups were weighed and examined postnatally (Narotsky et al.,1995). Three maternal and six developmental endpoints were evaluated. Several significant two-way interactions but no significant three-way interactions were observed. Maternal death exhibited no single chemical or main effects but DEHP and heptachlor were synergistic. For maternal weight gain on gestational days 6-8 main chemical effects were seen for all three agents as well as TCE-DEHP synergism and DEHP-heptachlor antagonism. Maternal weight gain on gestational days 6-20 adjusted for litter weight showed effects for TCE and heptachlor, but no interactions. Effects of all three agents were seen for full-litter resorptions and prenatal loss. The heptachlor effects were unexpected, particularly as seen with pooled data (heptachlor only plus heptachlor combinations) for each heptachlor dose, with 23% of 253 dams with resorbed litters at 8 mg/kg-d and 18% of 247 dams at 2.5 mg/kg-d vs. 12% in the controls. For full-litter loss, the TCE-heptachlor and DEHP-heptachlor interactions were antagonistic. For prenatal loss, the TCE-DEHP interaction was synergistic. Postnatal loss showed DEHP and heptachlor effects but no interactions. Analysis of pup weights on day 1 revealed TCE and DEHP effects and DEHP-heptachlor antagonism; on day 6 DEHP and heptachlor effects and DEHP-heptachlor antagonism,

and TCE-DEHP synergism were evident. The authors note that some antagonistic interactions of prenatal loss and full litter resorptions may reflect a ceiling effect and, based on heptachlor main effects, that heptachlor potentiated the other two agents. The authors thus regard all three two-way interactions to be synergistic for these related endpoints. Microphthalmia and anophthalmia incidences showed TCE and DEHP effects but no interactions.

Genning (1996) analysed a subset of the Narotsky et al. data to illustrate the use of ray designs in mixtures of chemicals. The selected response was prenatal loss. The rays selected were one for each single chemical and one mixture ray. The dose ratios for the rays were for (DEHP:heptachlor:TCE): (1:0:0); (0:1:0); (0:0:1); and (70:1:29). A threshold model was fitted along each of the four rays simultaneously. The author concluded that departure from additivity could not be claimed along the 70:1:29 ratio mixture ray.

Testicular toxicity

Studies on the testicular toxicity of DEHP have attempted to elucidate mechanisms of action, differences in sensitivity due to age and species and the potential for reversibility of toxic effects. Considerations of mechanism have focused on attempting to identify the active metabolite, and on the possible role of zinc depletion in DEHP-induced testicular toxicity. Results of both *in vivo* and *in vitro* experiments have tended to support the contention that MEHP, rather than DEHP itself, is the active testicular toxicant (Gray *et al*., 1982; Gray and Gangolli, 1986; Oishi, 1993; Sjoberg *et al*., 1986b).

Depletion of testicular zinc levels has been found to coincide with the occurrence of DEHP-induced testicular atrophy (Gray *et al*., 1982; Oishi, 1993; Oishi and Hiraga, 1983). Zinc supplementation, however, did not prevent testicular atrophy in DEHP-treated animals (Oishi and Hiraga, 1983). In general, clear evidence for a cause and effect relationship between zinc levels and the testicular effects of DEHP has not been reported (Oishi, 1993; Oishi and Hiraga, 1983).

Age-dependent differences in response to the testicular toxicity of DEHP are of particular importance in considering the issue of a sub-population which may be far more sensitive than the population at large. The peak period of sensitivity to the testicular toxicity of oral exposure to DEHP appears to be around the time of puberty (Dostal *et al*., 1988; Gray and Butterworth, 1980; Gray and Gangolli, 1986; Sjoberg *et al*., 1985c; Sjoberg *et al*., 1986b). Prenatal exposure alone, of either rats or mice, did not adversely affect eventual adult fertility (NTP, 1986; 1988, see discussion of these studies under "Developmental Toxicity"). There is no multigeneration reproductive toxicity study of DEHP available at present, however. Such a study, involving exposure of at least one generation from the prenatal period through at least one round of reproduction would allow evaluation of the reproductive effects of continuous exposure to DEHP.

Pathogenesis

A recent subchronic oral toxicity study conducted in rats produced evidence of DEHP-induced testicular toxicity at very low doses (Poon *et al*., 1997). For a treatment period of 13 weeks, groups of 10 male and 10 female Sprague-Dawley rats were administered diets which contained DEHP at concentrations of 0, 5, 50, 500 or 5,000 ppm. These concentrations were determined to have provided doses of 0, 0.4, 3.7, 37.6 or 375.2 mg/kg-day, respectively, to the male rats. No clinical signs of toxicity were observed and there were no changes in body weight gain or food consumption with DEHP exposure. At the high-dose both males and females showed significant

effects on liver and kidney weights. No specific effects on female reproductive organs were mentioned.

At the high-dose of 375.2 mg/kg-day, 9/10 males had atrophied seminiferous tubules, with complete loss of spermatogenesis and cytoplasmic vacuolation of the Sertoli cells lining the tubules. At the next lower dose (37.6 mg/kg-day), minimal Sertoli cell vacuolation was observed in 7/10 males. Because it was considered to represent an early change in the pathogenesis of adverse effects on the germ cells, the authors concluded that Sertoli cell vacuolation should be treated as an early adverse effect. Therefore, based on Sertoli cell vacuolation, the LOAEL for adverse effects on male rats in this study was determined to be 37.6 mg/kg-day and the NOAEL to be 3.7 mg/kg-day.

Age Dependence of Testicular Response

Gray and Butterworth (1980) found that DEHP-induced testicular atrophy was age-dependent in Wistar rats. Oral administration of 2,800 mg/kg-day for 10 days produced tubular atrophy in four-week-old rats, but had no effect on the testes of 15-week-old rats. In 4 and 10-week-old animals, advanced germinal cells were lost, with only spermatogonia, Sertoli cells and occasional primary spermatocytes remaining.

In a later study (Gray & Gangolli, 1986), four week-old rats given 2,800 mg/kg-day for 10 days showed significant reductions in body weight, testes weight, seminal vesicle weight and prostate weight. At the histological level, these animals also showed severe testicular atrophy, affecting virtually all tubules. Ten-week old animals given the same treatment showed no adverse effects on testes weights, but body weights and weights of accessory organs were significantly reduced. Up to 50% of seminiferous tubules were adversely affected. Fifteen-week-old animals had reduced body weights in response to DEHP treatment, but testicular parameters were unaffected. Additional experiments indicated that the testicular effects were more likely the result of direct action on the seminiferous tubules, than of indirect effects on hormonal levels. In particular, it was concluded that Sertoli cells are likely to be the primary target tissue of DEHP, as well as of other phthalates which cause testicular injury. Germ cells dependent on normally-functioning Sertoli cells would then be lost, in turn leading to tubular atrophy. These conclusions were supported by *in vitro* experiments indicating that MEHP is the active metabolite in DEHP-induced testicular toxicity, and *in vivo* experiments indicating that MEHP does not readily cross the intact blood-testis barrier provided by normally-functioning Sertoli cells. Thus it appears that the germ cells would not be directly exposed to MEHP unless the Sertoli cells are damaged, and the barrier they provide is disrupted.

Immature and adult rats were given five daily gavage doses of DEHP (Dostal *et al*., 1988). Testes were examined for toxicological effects at 24 hours following the last dose, and lasting effects on fertility were investigated in mating trials of sexually-mature animals. Suckling pups (aged one, two, or three weeks of age at the commencement of treatment) were unable to survive doses of 2,000 mg/kg, and had reduced relative testes weights at 1,000 mg/kg dose level. Six-week-old rats survived either dose level, with reduced relative testes weights. Twelve-week old rats were unaffected at the lower dose, but had reduced testes weights at the higher dose. Male rat pups exposed to 1,000 mg/kg DEHP on postnatal days 6 to 10 had reduced numbers of Sertoli cells as measured 24 hours following the last dose. Sertoli-cell numbers for these animals were normal at 6 and 13 weeks of age. Decreases in testes weights and testicular spermatid numbers were evident at 13 and 19 weeks of age, but not at 11, 12, 16 or 23 weeks of age. When these males rats were mated with untreated females rats, no consistent changes in fertility, implantation rate or numbers of live fetuses were detected. The results of this study generally confirmed the Sertoli cell as the

primary testicular target cell for orally-administered DEHP, as well as the higher sensitivity of younger animals.

Age-specific responses were found when DEHP was given in the diet to male rats at doses of 1,000 or 1,700 mg/kg for 14 days (Sjoberg *et al*., 1986b). Animals were 25, 40 or 60-days-old at the beginning of treatment. Body weight gain was retarded in all groups, and testicular weight was markedly reduced in younger rats given the higher dose of DEHP. At the histological level, tubular damage was evident in the two younger groups of treated animals, all tubules being affected in the 25-day-old animals. The oldest group of animals showed resistance to the testicular toxicity of DEHP. Pair-fed controls exhibited only minor changes in testicular parameters, indicating a direct toxic effect of DEHP and/or its metabolites.

Similar results were obtained in a further experiment, using a similar protocol, but where DEHP was given by gavage (Sjoberg *et al*., 1986b). Again, the greatest response to treatment was shown by animals in the youngest age group (25-days-old at the onset of the experiment), while 60-day-old animals were unaffected. Determinations of blood and urine levels of DEHP and MEHP demonstrated far more circulating MEHP in the younger animals. A third experiment, employing iv infusions of DEHP at a dose of 500 mg/kg, did not indicate a decrease in testicular sensitivity with increasing age (Sjoberg *et al*., 1986b). Only 25-day-old and 40-day-old rats were tested under this protocol. These results were consistent with those of an earlier study (Sjoberg *et al*., 1985c) and support the authors' proposal that the age-related differences in testicular response to orally administered DEHP may be, at least partially, due to age-dependent differences in the pharmacokinetics of DEHP.

Species and Strain Differences in Response

Mice, hamsters and marmosets are generally less sensitive than rats to the testicular toxicity of DEHP (Gray *et al*., 1982; Rhodes *et al*., 1986). There are also differences between mouse strains in the degree of responsiveness to this chemical (Oishi, 1993). Gray *et al*. (1982) compared the extent of phthalate ester-induced testicular toxicity in rats and hamsters. Both DEHP and MEHP caused reductions in testes weights and increases in tubular atrophy in rats, but only MEHP had any effect on hamsters. A possible explanation for these findings was a difference in the relative rates of intestinal hydrolysis of DEHP to MEHP which was significantly slower in hamsters. Additionally, the concentration of zinc was decreased in the testes and increased in the urine of rats, but not hamsters, treated with DEHP. It could not be determined from this study, however, whether testicular zinc depletion was a cause or an effect of testicular injury.

Among mouse strains, Crj:CD-1 mice were found to be far more sensitive than Jcl:ICR mice to the testicular toxicity of DEHP (Oishi, 1993). The finding of significantly higher blood concentrations of MEHP in DEHP-treated Crj:CD-1 mice than in Jcl:ICR mice, supports the importance of pharmacokinetic variables in determining responsiveness. Conversely, testicular zinc concentrations were depleted by DEHP in a dose-dependent fashion in both strains, a finding which does not support a zinc-mediated mechanism for the testicular toxicity of DEHP.

Reversibility of Testicular Effects

Agarwal *et al*. (1986) exposed male F344 rats to 0, 320, 1,250, 5,000 or 20,000 ppm DEHP in the diet for 60 days. A dose-dependent reduction in total body weight, testis, epididymis and prostate weights occurred at the two highest dose levels. At 20,000 ppm, degenerative changes, decreased zinc content, reduced epididymal sperm density and motility and increased abnormal sperm were found in the testis. On day 61, the treated males were returned to a normal diet and mated with

untreated females. The incidence of pregnancy, mean litter weight, frequency of stillbirths and neonatal deaths and mean litter growth up to seven days of age were unaffected by DEHP treatment. However, litter size was significantly reduced in the 20,000 ppm group. Cessation of exposure resulted in partial to complete recovery from toxicity, with the gonads recovering more slowly than other systems.

Young, adult male Crj:Wistar rats were given 2,000 mg/kg daily, by gavage, for 14 days (Oishi, 1985). Treated and control animals (20 per group) were evaluated at either 10 or 45 days following the cessation of the treatment period. Body weight gain of treated animals was slightly depressed during the dosing period, but gradually recovered once treatment ceased. At one day following the end of treatment, liver weights of DEHP-treated rats were significantly greater than those of controls. Testicular weights, and the weights of accessory sex organs were significantly reduced in treated animals. Testicular histology revealed severe DEHP-induced damage to the seminiferous tubules. Testicular zinc levels were significantly reduced in treated animals. While testicular testosterone levels were significantly higher in treated than control animals, the opposite was the case for serum testosterone. At 45 days following the cessation of DEHP exposure, of the organ weights determined, only testicular weights had not returned to control levels. Testicular histopathology was still abnormal, testicular zinc concentrations were still reduced and testicular testosterone concentrations were still elevated. The author concluded that the potential for reversibility of DEHP-induced testicular atrophy is limited.

Ovarian Toxicity

Treatment with DEHP at a dose of 2,000 mg/kg, suppressed serum estradiol levels and ovulation (Davis *et al*., 1994). Adult female Sprague-Dawley rats were given DEHP, by gavage, once per day for 1 to 12 days. Estrus cycles of treated animals were prolonged and, even when vaginal estrus did occur, ovulation was not necessarily a co-occurrence. Analysis of serum hormone levels and of ovarian histopathology indicated that DEHP altered the function of pre-ovulatory follicle granulosa cells. Normally, these cells produce estradiol which, when released into the peripheral circulation, triggers the ovulatory surge of LH in female rats; the same hormonal control mechanism operates in women. This proposed mechanism for the female reproductive toxicity of DEHP is consistent with the findings of infertility in treated female mice (NTP, 1982).

Lactation

Both DEHP and MEHP were identified in the milk of lactating rats given DEHP by the oral route (Dostal *et al*., 1987a). Oral doses of 2,000 mg kg body weight were given daily on lactation days 2 to 6, 6 to 10 or 14 to 18. Decreased body weights in both treated maternal animals and their offspring were thought to be a nonspecific result of decreased feed consumption; pair-fed (restricted diet) controls showed the same effect. However, DEHP treatment did specifically lead to significant increases in the hepatic peroxisomal enzymes palmitoyl CoA oxidase and carnitine acetyltransferase in both dams and pups. Direct measurements were made of DEHP and MEHP levels in milk, as well as in maternal and pup plasma. For DEHP, the milk to plasma ratio was greater than 200, suggesting a very efficient extraction mechanism for DEHP.

Genetic Toxicity

DEHP

DEHP has tended to be negative in *in vivo* and *in vitro* genotoxicity assays (Budroe and Williams, 1993); only nine positive tests were noted in a list of 100 in the Genetic Activity Profile (GAP) (U.S. EPA, version 4.06, 1994).

In vitro Genotoxicity Assays

DEHP has been found to be nonmutagenic in at least eight separate *Salmonella*/mammalian microsomal mutation assays (Budroe and Williams, 1993). Tomita *et al*. (1982) have reported positive results; however, the data were not convincing. Results of only a single dose were reported, and the increase was less than two-fold. DEHP does not induce mitotic gene conversion or gene mutations, but does induce aneuploidy in *Saccharomyces cerevisiae* (Ashby *et al*., 1985).

Mixed results, mostly negative, have been obtained in the L5178Y mouse lymphoma mutation assay, with only 1 of 10 investigators reporting a positive response in a collaborative study (Ashby *et al*., 1985). Tests with cultured CHO cells found that DEHP does not increase chromosomal aberrations in the presence (Galloway *et al*., 1985) or absence (Phillips *et al*., 1982; Galloway *et al*., 1985) of liver S9 prepared from Aroclor 1254-induced rats. However, Tsutsui *et al*. (1993) found that DEHP induced chromosomal aberrations in Syrian hamster embryo (SHE) cells in the presence of exogenous metabolic activation (rat liver S9). In addition, DEHP did not induce chromosomal aberrations but did induce aneuploidy in a fibroblast cell line (CH1-L) derived from Chinese hamster liver which is claimed to have metabolic activation capability (Danford, 1985). Other studies with CH1-L cells found that DEHP caused a dose-dependent decrease in the anaphase-telophase/metaphase ratio and an increase in the number of chromosome cluster groups and abnormal division stages, indicating an induction of spindle damage (Parry, 1985). DEHP did not induce any increase in sister-chromatid exchanges (SCE) in CHO cells (Douglas *et al*., 1986) or human peripheral lymphocytes (Obe *et al*., 1985) with or without exogenous metabolic activation.

DEHP-induced cellular transformation has been studied in a number of systems. DEHP was negative in the BALB/3T3 assay with and without rat primary hepatocytes (Astill *et al*.,1986). Alternatively, the majority of transformation assays in the IPCS collaborative study were positive for DEHP (Ashby *et al*., 1985). Positive results have been obtained by other investigators using SHE cells, embryonic mouse fibroblasts, retrovirus infected Fischer rat embryo cells (WHO,1992) and SHE cells (Tsutsui *et al*., 1993). Cell transformation of SHE cells was enhanced by exogenous metabolic activation using rat liver S9. DEHP was observed to inhibit intracellular communication (IC) in V79 cells at nontoxic concentrations of 10 to 30 mg/L (Malcolm & Mills, 1989). Alternatively, Mikalsen and Sanner (1993) observed that DEHP transformed SHE cells had the same ability of intercellular communication as normal cells suggesting that decreased IC is neither sufficient or necessary to induce morphological transformation of SHE cell colonies.

In vivo Genotoxicity Assays

Tomita *et al*. (1982) reported increased chromosomal aberrations and transformation in embryonic cells from Syrian hamsters treated transplacentally with DEHP at 3.75 to 15 g/kg administered by maternal gavage. However, the proportion of normal diploid cells in all cultures including the

controls was low, suggesting chromosomal instability or technical deficiencies in experimental procedures. Singh *et al*. (1974; 1975) and Autian (1982) tested an *in vivo* system, and found weakly positive results in dominant lethal assays in mice. Interpretation of effects in these studies was difficult because mice treated at the high dose may have experienced reduced fertility as a result of testicular degeneration, and effects seen at lower doses were not statistically significant. DEHP was also negative in the sex-linked recessive lethal test and other genotoxic endpoints in *Drosophila melanogaster* (WHO, 1992).

DNA Adducts

Albro *et al*. (1982) reported association of radioactivity with DNA when 2-ethyl-labeled [^{14}C]-DEHP was administered orally to rats but not when carbonyl-labeled DEHP was administered. Von Daniken (1984) found similar association of labeled DEHP with DNA under comparable conditions, but also found label in DNA when [^{14}C]-ethylhexanol was given orally. These results suggest that DEHP undergoes metabolism before incorporation into nucleotides rather than covalently binding to DNA as typically seen with genotoxic carcinogens. No DNA adducts were found in rat liver DNA following administration 2,000 mg/kg for three days using the ^{32}P-post-labeling technique (Gupta *et al*., 1985).

Oxidative DNA Damage

Male F344 rats exposed to 1.2% DEHP in the diet for one or two weeks did not show significant increases in 8-hydroxydeoxyguanosine (8-OH-dG) kidney DNA adducts, but did show statistically significant increases in 8-OH-dG liver DNA adducts at both time points (Takagi *et al*., 1990). However, the observed increase was less than two-fold greater than control.

MEHP

In vitro Genotoxicity Assays

A dose-dependent increase in toxicity to rec$^-$ (DNA repair deficient) compared to rec$^+$ was reported in *Bacillus subtilis* by Tomita *et al*. (1982). These authors also reported a dose-dependent increase in revertants in *Salmonella* (TA 100) and *Escherichia coli* treated with MEHP in suspension.

A small increase in HGPRT mutations in V79 cells treated with MEHP was reported by Tomita *et al*. (1982). According to Turnbull and Rodricks (1985), the Tomita report lacks procedural details and supporting data, making a detailed evaluation of the data impossible. Philips *et al*. (1982) examined the ability of MEHP to induce mutations in CHO cells at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus. Two studies were done, using concentration ranges of 0.37 to 1.25 mM and 0.08 to 1.25 mM, respectively. A significant increase (two-fold greater than control) was seen at the lowest concentration (0.37 mM) in the first study; the results at all other concentrations in both studies were negative. MEHP was found to be negative in the L5178Y mouse lymphoma mutation assay by Kirby *et al*. (1983).

Tomita *et al*. (1982) found that MEHP induced increased SCE in V79 cells treated for 24 hours with 25 or 50 µg/mL MEHP. Both dose groups displayed increased SCE, with a two-fold increase compared to control noted in the 50 µg/mL dose group. Phillips *et al*. (1982) reported no increase in SCEs but did find an increased frequency of chromosomal aberrations in CHO cells treated for two hours with MEHP at 0.8 to 1.75 mM. These dose levels reduced cell survival to between 65 to less than 10% of control values. MEHP treatment for two hours induced chromosomal damage

in both CHO and rat liver cells in a dose-dependent manner (Phillips *et al*., 1986). In CHO cells, the clastogenicity of MEHP was unaffected by the presence of an exogenous metabolic activation system (S9 mix). Negative results with 2-ethylhexanol and *o*-phthalic acid (Von Daniken *et al*., 1984) suggest that these compounds were not responsible for the clastogenicity of MEHP.

Tsutsui *et al*. (1993) observed morphological transformation of SHE cells treated with MEHP for 48 hours.

In vivo Genotoxicity Assays

A dose-dependent increase in chromosome aberrations and transformation was observed in Syrian hamster embryo cells treated transplacentally at 375 to 1,500 mg/kg MEHP (Tomita *et al*., 1982).

DNA Adducts

Albro *et al*. (1982) found an association of label with DNA when [^{14}C]-MEHP was administered orally to rats. Labeling was less than that which resulted with an equivalent amount of [^{14}C]-DEHP. Although the nature of this association is uncertain, the study by Von Daniken *et al*. (1984) with DEHP suggests that incorporation of label via intermediary metabolism is possible.

2-Ethylhexanol

The only suggestion of positive results with 2-ethylhexanol comes from a study where a slight increase in azaguanine-resistant mutants in *Salmonella* treated with 0.5 to 1.5 mM 2-ethylhexanol was found (Seed, 1982). The increase in frequency of mutants/survivor was small, and was matched by a reduction in survival, such that there was no increase in the absolute number of mutants at any dose. Bone marrow cells from male F344 rats exposed to 0.02, 0.07 or 0.21 mL/kg-day 2-ethylhexanol did not show increases in chromosomal aberrations or any induction of aneuploidy (Philips *et al*., 1982).

Carcinogenicity

NTP Cancer Bioassay

The following summary evaluation of the potential carcinogenicity of DEHP is reproduced from U.S. EPA's Carcinogen Assessment Group (CAG) November 1986 Review:

> "The carcinogenicity of DEHP has been tested in a bioassay using B6C3Fl mice and Fischer 344 rats (NTP, 1982; Kluwe *et al*., 1982). Groups of 50 animals of each sex of each species were fed diets containing DEHP at two dose levels or untreated diet (controls) for 103 weeks. Diets containing 6,000 or 12,000 mg/kg feed were ad-ministered to rats, while mice received 3,000 or 6,000 mg/kg feed. A one to two week non-treatment period was allowed after the exposure period, after which survivors were sacrificed and examined grossly and microscopically. Histopathological analyses were conducted on all animals killed or discovered dead (without excessive tissue damage) and included examination of skin, lungs, bronchi, trachea, larynx, bones, bone marrow, spleen, lymph nodes, heart, liver, pancreas, esophagus, stomach, small intestine, large intestine, kidney, bladder, pituitary, adrenal, thymus, thyroid, parathyroid, salivary gland, mammary gland, testis or ovary, prostate and seminal vesicles or uterus and brain.

"In rats, body weight gain was decreased in males treated at both dose levels and females treated at 12,000 mg/kg diet. Food consumption was slightly decreased in all groups of treated rats compared to controls. Daily doses of DEHP were calculated to be 322 and 674 mg/kg body weight for low and high-dose males and 394 and 774 mg/kg body weight for low and high-dose female rats, respectively. Survival of the male or female treated rats was not affected by DEHP at either dose level. About 60% of the male rats (including treated and control groups) and 70% of the female rats survived until the end of the study at 105 weeks. Histopathological examination identified non-neoplastic seminiferous tubular degeneration and testicular atrophy among 90% (43/48) of the rats exposed to 12,000 mg/kg diet; only 1 of 49 control rats exhibited this pathology. Twenty-two of the 49 high-dose males had hypertrophied anterior pituitary, and hepatic changes (clear cellularity) were increased in a dose-dependent manner in males. Only a slight increase in hepatic clear cell change was noted in treated female rats.

"The incidence of neoplastic lesions of the liver was increased in both male and female rats exposed at 12,000 mg/kg diet. Twelve of the 49 high-dose males had hepatocellular carcinomas or hepatic neoplastic nodule ($P < 0.05$), while only 3 of 50 control males had hepatic neoplasia. In high-dose females, 13 of 50 animals had hepatocellular carcinoma or neoplastic nodule ($P < 0.001$), while none were identified among controls. Both male and female rats showed a significant ($P < 0.01$) dose-dependent increase in the incidence of liver neoplasms. The incidence of neoplasms of the pituitary, thyroid and testis of male rats showed a significantly ($P < 0.05$) decreasing dose-dependent trend (Kluwe *et al*., 1982).

"In mice, body weight gain was slightly decreased in females ingesting DEHP, while males gained weight normally. Food consumption was not decreased in any group of treated mice. At the two dose levels tested (6,000 and 3,000 mg/kg diet), daily doses of DEHP were 1,325 and 672 mg/kg for males and 1,821 and 799 mg/kg for females, respectively. Histopathological examination of all mice revealed a higher incidence of seminiferous tubule degeneration and chronic renal inflammation (non-neoplastic abnormalities) among high-dose males than control males. The incidence of hepatocellular carcinoma was increased significantly ($P < 0.05$) in both male (19/50) and female (17/50) high-dose groups compared to controls (9/50 males; 0/50 females). A significant ($P < 0.05$) dose-dependent trend of increasing hepatocellular carcinoma was also demonstrated for treated male and female mice. Metastases of the hepatocellular carcinomas were observed in the lungs of 12 males and 8 females treated with DEHP. The incidence of neoplastic lesions of other sites was not increased in any group of treated mice compared to controls (Kluwe *et al*., 1982).

"The validity of the carcinogenic effect of DEHP determined in the NTP (NTP, 1982; Kluwe *et al*., 1982) bioassay using rats and mice was questioned by Northrup *et al*. (1982). The high level of exposure was criticized as being excessive, but at the levels where carcinogenesis was indicated, toxic effects were minimal and normal lifespan of the animals was not shortened. The doses administered were therefore near or below the level regarded as the maximally tolerated dose (MTD) (Kluwe *et al*., 1983). Northrup *et al*. (1982) also questioned the incidence and variability of spontaneous liver tumors and suggested that frequencies occurring in DEHP-treated rats and mice were not greatly increased. Kluwe *et al*. (1983) supported the significance of the increased incidence of hepatocellular carcinoma among treated mice and rats with historical control data which actually strengthened the statistical significance of the tumor incidence. The decreased incidence of tumors in endocrine organs among male rats, viewed suspiciously by

Northrup *et al*. (1982), apparently resulted from atrophy of these organs and not through a protective effect. Differences in the ability to conjugate metabolites of DEHP between primates and rodents were regarded as possible reasons to consider rodents a poor model for carcinogenic tests for humans. Kluwe *et al*. (1983) commented that there is no evidence that conjugates of DEHP metabolites are the effective agent and that the parent compound or its primary metabolite MEHP occurs in rodents and primates and may be the etiologic agent. Phillips *et al*. (1982) reported that MEHP caused chromosome damage in Chinese hamster ovary (CHO) cells and may cause the carcinogenic effect of DEHP. In short, the NTP (1982) study was considered valid in design and conclusions and showed sufficient evidence to consider DEHP a liver carcinogen in rats and mice (Kluwe *et al*., 1983)."

Other Cancer Bioassays

Carpenter *et al*. (1953) administered 0.04 and 0.4% DEHP in the diet for two years to 192 Sherman rats. Nine treated rats were reported to have neoplasms (which were not characterized pathologically); this tumor incidence was not considered by the investigators to be significant. In a later study, Harris *et al*. (1956) administered DEHP in the diet to 172 Albino-Wistar rats for two years at dose levels of 0.1 or 0.5%. A total of three fibroliposarcomas were observed, and were considered by the investigators to be unrelated to the treatment. Few treated or control animals were reported to have survived the total two-year testing period. Both the Carpenter *et al*. (1953) and Harris *et al*. (1956) two-year bioassays reported no carcinogenic effects from dietary administration of DEHP. However, these studies do not conform to current standards for carcinogenicity bioassays, and were probably insufficiently sensitive to have detected an effect of DEHP of the small magnitude seen in the NTP bioassay.

Two recent industry-sponsored bioassays in rodents were conducted by Corning Hazelton (1996). The mouse study was not available in sufficient detail to allow adequate review. In the 104 week study in F-344 rats the animals (65/sex/dose) were administered 0, 100, 500, 2,500 or 12,500 ppm DEHP in the diet for 104 weeks with an additional 12,500 ppm dose group exposed for 79 weeks, followed by a 26-week recovery period. Mortality, clinical observations, body weight, food consumption, clinical pathology, organ weights, necropsy and histopathology were evaluated. Also at 1, 2, 13, 79 and 104 weeks chemically-induced cell proliferation and peroxisome proliferation in the livers of control and high-dose groups were evaluated. At 100 ppm (5.8 mg/kg-day male and 7.3 mg/kg-day female) and 500 ppm (28.9 and 36.1 mg/kg-day) there were no treatment-related effects including DEHP-induced liver peroxisome proliferation. At 2,500 ppm significantly increased mean liver weights occurred in both sexes with DEHP induction of liver peroxisome proliferation evident in both sexes at 104 weeks. In rats killed at study termination, hepatocellular adenomas were detected in 16% and 2% of the males and females, compared to 9% and 0% in controls, respectively. For all deaths, the total incidence of monocellular leukemia was increased to 49% (32/65) in males vs. 23% (15/65) in controls. At 12,500 ppm an increased incidence of adenomas and carcinomas were first detected at week 79. DEHP induction of liver peroxisome proliferation was evident in both sexes at weeks 1, 2, 13 and 104. In rats killed at study termination, hepatocellular adenomas were detected in 45% and 8% of males and females compared to 9% and 0% in controls, respectively. Hepatocellular carcinomas were detected in 43% and 28% in males and females compared to 2% and 0% in controls, respectively. For all deaths, the incidence of monocellular leukemia was increased to 42% (27/65) compared to 23% (15/65) in control males. In the last dose group (12,500 ppm for 78 weeks), there was evidence that some treatment-related effects were reversible or did not progress following cessation of exposure. Treatment-related liver enlargement and liver peroxisome proliferation appeared to be reversible as

were some liver histological effects. In rats killed at study termination hepatocellular adenomas and carcinomas were seen in 25/45% of males and 13/8% of females compared with 45/28% and 8/28%, respectively, in the 12,500 ppm dose group without a recovery period.

A second study in B6C3F1 mice was not available for review but was summarized in a report submitted to the Office of Environmental Health Hazard Assessment (OEHHA) by Wilkinson (1997). Fifty animals/sex/dose group were exposed to 0, 100, 500, 1,500 or 6,000 ppm DEHP in the diet. The study identified NOELs for peroxisome proliferation of 19.2 mg/kg-day in males and 23.8 mg/kg-day in female mice. Increased liver weights were seen at 500 ppm DEHP. Increased incidences of hepatocellular adenomas and carcinomas were seen at 1,500 and 6,000 ppm DEHP. The tumor incidence for females was 3/36, 2/32, 3/40, 3/39 and 8/35 and for males was 2/42, 4/38, 4/39, 9/41 and 5/19 for the respective increasing doses. The tumors were not specified but presumably are liver carcinomas.

U.S. EPA Evaluation

U.S. EPA (IRIS, last revised 2/1/93) classifies DEHP as a probable human carcinogen based on dose- dependent liver tumor responses in both sexes of rats and mice. They use the most sensitive response in male mice as the basis of their carcinogenic slope factor of 1.4×10^{-2} $(mg/kg\text{-}day)^{-1}$. U.S. EPA considers the human carcinogenicity data inadequate. Supporting data for carcinogenicity include the finding that the MEHP monoester metabolite of DEHP exhibited DNA damage and point mutations in microbial assays and clastogenic activity in mammalian cells *in vitro*. Both DEHP and MEHP induced chromosomal aberrations and morphological transformations in cultured Syrian hamster cells exposed *in utero*.

IARC Evaluation

IARC reviewed and evaluated DEHP for carcinogenicity in 1982. The IARC working group concluded that there is sufficient evidence for the carcinogenicity of DEHP in mice and rats, based on the 1982 NTP cancer bioassay. There was no adequate epidemiological study available to the working group. IARC classifies DEHP as a Group 2B carcinogen (IARC, 1982).

DOSE-RESPONSE ASSESSMENT

Carcinogenicity

Possible Modes of Action

DNA Binding

Albro *et al*. (1982) found that carbonyl-labeled DEHP did not bind to purified protein, DNA or RNA from rat liver *in vivo*. Ethyl-[1-^{14}C] -hexyl-labeled DEHP and MEHP bound strongly with purified DNA, but the binding was not demonstrated to be covalent. Label from free [^{14}C]-2-EH failed to bind with DNA.

Lutz (1986) also looked into whether or not DEHP could bind covalently to rat liver DNA. DEHP radiolabeled in various positions was administered orally to female F344 rats with or without

pretreatment for four weeks with 1% unlabelled DEHP in the diet. After 16 hours, DEHP with [^{14}C]-and [^{3}H]-labels in the alcohol moiety, as well as 2-ethyl-[1-^{14}C]hexanol, resulted in radioactivity in isolated liver DNA. HPLC analysis of enzyme-degraded DNA showed that the normal nucleosides had incorporated radiolabel whereas no radioactivity was measured in fractions where the carcinogen-modified nucleoside adducts were expected. Quantitative evaluation of the data, using a covalent binding index (µmole chemical bound per mole DNA nucleotides)/(mmole chemical administered per kilogram body weight), indicated that covalent interaction with DNA is highly unlikely to be the mechanism of tumorigenesis by DEHP in rodents.

Initiation

Peroxisome proliferating agents such as DEHP which lack direct genotoxicity or binding to liver DNA have recently been subject to initiation studies. Garvey *et al*. (1987) administered 10 g/kg DEHP to male F-344 rats by gavage at either 6, 12 or 24 hours. After two weeks of recovery on a base diet, rats were given two weeks of a diet containing 0.02% 2-acetylaminofluorene (AAF). At midpoint of the AAF regimen, rats received a single oral dose of 1.5 mL/kg carbon tetrachloride ($CC1_4$). This sequence of AAF-$CC1_4$ treatment is known to cause the selective growth of altered hepatic foci (AHF) initiated with a broad class of compounds. One week after the end of the AAF feeding period, hepatic foci were quantified using six histological markers. DEHP did not initiate AHF. Since one current hypothesis for the mechanism of carcinogenic action of DEHP is based on the central role of peroxisomes, a second initiation experiment was devised which allowed the induction of peroxisomes during the initiation phase (Garvey *et al*., 1987). Rodents received 1.2% DEHP in the diet for 12 weeks. Following the 12 weeks of DEHP treatment, the DEHP diet was removed and replaced by a diet containing 0.05% phenobarbital for weeks 13 through 52. As in the previous initiation experiment, livers were evaluated for AHF. The results failed to demonstrate a differential response in that the groups receiving the DEHP initiation did not have an increased number of foci compared to the promotion controls receiving only phenobarbital.

Ward *et al*. (1986) administered male B6C3Fl mice a single dose of 25 or 50 g/kg DEHP by gavage followed by 6 or 18 months of a diet containing 500 ppm phenobarbital. Under the above conditions, DEHP did not induce AHF or carcinomas in the animals.

Promotion

A number of studies have tested the ability of DEHP and other peroxisome proliferators to accelerate the progression of preneoplastic cells to cancer. Since positive staining for γ-glutamyltranspeptidase has been determined to be a poor marker for peroxisome proliferator-induced lesions (Rao *et al*., 1987; Glauert *et al*., 1986), studies using this staining method as a marker are considered inadequate. Popp *et al*. (1985) administered male F-344 rats a single injection of diethylnitrosamine (DEN) followed two weeks later by six months of 1.2% DEHP in the diet. DEHP did not increase the size or number of AHF using six histological markers. Rats were administered 200 ppm of the initiator N-2-fluorenylacetamide for seven weeks in the diet, followed by four weeks of recovery on the base diet and 24 weeks on a diet containing 1.2% DEHP (Williams *et al*., 1987). DEHP did not increase the size or number of AHF identified by iron exclusion or hematoxylin-eosin staining. DEHP also failed to increase the incidence of liver tumors, although the number of rats studied was low (N = 6). Preat and Roberfroid (1987) administered male Wistar rats a single injection of DEN followed five weeks later by 10 months of 1% DEHP in the diet. DEHP did not increase the incidence of liver cancer in this experiment.

Ward *et al*. (1983) administered B6C3Fl mice a single dose of DEN followed by up to six months of a diet containing 0.3, 0.6 or 1.2% DEHP. At 0.6 and 1.2%, DEHP increased both the number

and size of AHF and the tumor incidence. In a later study, Ward *et al*. (1984) administered mice a diet containing 0.03% DEHP for short periods after a single dose of DEN. DEHP enhanced the incidence of AHF in mice after only 28 days. Therefore, the two studies by Ward *et al*. demonstrate the DEHP promotion activity in mice.

Several peroxisomal proliferators with more potent hepatocarcinogenic activities have been tested for promotion activity in rats. Chronic feeding studies with clofibrate have been shown to increase the incidence of hepatic tumors after DEN initiation (Preat and Roberfroid, 1987). Nafenopin in the diet also increases the incidence of tumors following DEN initiation (Preat and Roberfroid, 1987). Glauert *et al*. (1986) reported that WY-14,643 enhanced the formation of adenosine triphosphatase- and glucose-6-phosphatase-deficient foci after initiation by DEN. Thus, these other peroxisomal proliferating compounds appear to promote liver cancer in rats. Investigators speculate that promotion experiments with DEHP may require high doses of DEHP (1.2% or greater in the diet) and large groups of rats to detect a significant promoting effect (Conway and Butterworth, 1988).

Genotoxicity Secondary to Peroxisomal Generation of Hydrogen Peroxide

The carcinogenicity of nonmutagenic and non-DNA adduct-forming peroxisome proliferators has been proposed to be related to biologically active products of the proliferated peroxisomes rather than a direct chemical effect. The hypothesis that there is a relationship between peroxisome proliferation and liver carcinogenesis in rodents was proposed by Reddy *et al*. (1980) on the basis of their findings with a group of five drugs used in the treatment of hyperlipidemia. These chemicals caused hypolipidemia, hepatomegaly, proliferation of liver peroxisomes and hepatocellular carcinoma in mice or rats, but none caused DNA damage or were mutagenic in the Ames assay. Peroxisomes contain several enzymes that generate hydrogen peroxide (H_20_2) and catalase, which degrades H_20_2 to H_20 and 0_2. Treatment of rats with DEHP (Conway and Butterworth, 1988) causes a large and persistent induction of the H_20_2-producing enzyme fatty acyl-CoA oxidase, the first enzyme in the peroxisomal beta-oxidation system. In contrast, DEHP does not induce catalase activity to the same degree as acyl-CoA oxidase. DEHP also produces substantial decreases (30 to 70%) in the activity of glutathione peroxidases and glutathione-S-transferases, the enzymes responsible for H_20_2 and hydroperoxide degradation, respectively. Rao and Reddy (1987) and others have hypothesized that an imbalance between H_20_2 production and degradation could lead to leakage of H_20_2 from the induced peroxisomes. Cytoplasmic H_20_2 could then interact with iron and initiate lipid peroxidation via Fenton reactions. Products of lipid peroxidation such as aldehydes and hydroperoxides have been shown to damage DNA (Ochi and Cerutti, 1987; Hornsby and Harris, 1987).

A number of studies have reported an accumulation of lysosomes containing lipofuscin in rodent hepatocytes after chronic treatment with DEHP and other peroxisome proliferators. Mitchell *et al*. (1985) administered 1,000 mg/kg-day DEHP (1%) in the diet of rats and observed an increase in periodic acid Schiff base-positive, diatase-resistant granules in hepatocytes after one month. Conway *et al*. (1988) administered male F-344 rats a diet containing 1.2% DEHP and reported statistically significant increases in lipofuscin after 40 days. Conjugated dienes, another product of lipid peroxidation, have also been assayed in livers of rats administered peroxisome proliferators. Studies with DEHP have shown that it takes six months or more of treatment for accumulation of conjugated dienes to occur (Lake *et al.*, 1987).

There have been several attempts to correlate genotoxicity with the production of active oxygen species in hepatocytes. Butterworth *et al*. (1984) and Kornbrust *et al*. (1984) administered 1.2 and 2.0% DEHP, respectively, to male rats for one month to induce peroxisome proliferation.

Kornbrust *et al*. (1984) also inhibited catalase activity *in vivo* to maximize potential H_20_2- induced DNA damage. DEHP treatment did not cause an increase in unscheduled DNA synthesis (UDS) in any of the above experiments. However, experimental evidence has shown that UDS is not a very sensitive assay for detecting DNA damage induced by H_20_2 or lipid peroxidation products. Butterworth *et al*. (1984) and Elliot and Elcombe (1987) treated rats with 1.2% DEHP in the diet for one month and 2 g/kg-day by gavage for 20 to 30 days, respectively. In both experiments DEHP treatment did not cause a detectable increase in DNA strand breaks in isolated hepatic nuclei. It is noted that H_20_2 induced strand breaks have been shown to repair very rapidly, and that this may have occurred during the preparation of the DNA.

DEHP has also been demonstrated to cause modest (less than two-fold greater than control) but statistically significant increases in 8-hydroxydeoxyguanosine (8-OH-dG) liver DNA adducts (Takagi *et al*., 1990). Unrepaired 8-OH-dG adducts may lead to base mispairing, resulting in genotoxicity (Clayson *et al*., 1994).

Attempts have been made to determine the relationship between the degree of peroxisome proliferation and the relative hepatocarcinogenicity of several peroxisome proliferators. Reddy *et al*. (1986) administered male F-344 rats diets containing ciprofibrate, DEHP and di(2-ethylhexyl)-adipate for 30 days and compared hepatic peroxisomal fatty acyl-CoA oxidase activity to historical carcinogenesis data. A dose of ciprofibrate (0.02%) that caused a 100% incidence of carcinomas after 60 weeks can be compared to doses of DEHP (1.2%) and di(2-ethylhexyl)adipate (2.5%) that caused less than a 10% incidence of carcinomas after two years. In contrast to the relative carcinogenicities of the above diets, fatty acyl-CoA oxidase activity in rats fed ciprofibrate was only double that of the rats receiving DEHP or di(2-ethylhexyl)adipate.

Tomaszewski *et al*. (1986) administered male F-344 rats and female B6C3Fl mice nafenopin, DEHP or di(2-ethylhexyl)adipate for 14 days. Steady-state H_20_2 concentrations were measured in liver homogenates. In rats the rank order of calculated H_20_2 concentrations and the carcinogenicity of the compound was similar (i.e., nafenopin > DEHP > di(2-ethylhexyl)adipate). However, whereas the concentrations of H_20_2 in homogenates were five-fold higher in mice treated with DEHP than mice treated with di(2-ethylhexyl)adipate, these two chemicals show equivalent carcinogenic potencies in mice. Marsman *et al*. (1987) and Conway *et al*. (1988) measured peroxisome proliferation in the livers of male F344 rats fed 1.2% DEHP and 0.1% WY-14,643 for up to one year. After one year, rats treated with WY-14,643 exhibited approximately 60 neoplasms per liver compared to zero found in DEHP-fed or control rats. Although peroxisome proliferation was similar in rats receiving DEHP or WY-14,643, fatty acyl-CoA oxidase activities and peroxisomal volume densities were 20 to 30% greater in rats fed WY-14,643. Data from the above studies indicates that the differences in peroxisomal induction are marginal at best, and do not reflect the difference in the carcinogenic response seen in rats fed these peroxisomal proliferating chemicals.

The relationship between accumulation of lipofuscin and conjugated dienes in the rat liver and carcinogenicity was examined by Conway *et al*. (1988). Rats received 1.2% DEHP or 0.1% WY-14,643 in the diet for one year, with many liver tumors occurring from WY-14,643 only. DEHP produced a two- to four-fold increase in lipofuscin after 40 to 365 days of treatment. With WY-14,643, lipofuscin increased after only 18 days and continued to reach values about 30-fold above controls after a year. Conjugated dienes were increased after 6 or 12 months of WY-14,643 feeding, whereas DEHP feeding did not increase conjugated dienes after a year. An earlier study (Lake *et al*., 1987) found that two years of DEHP feeding increased conjugated dienes in rat livers. The Conway *et al*. (1988) study suggests a possible general correlation between lipofuscin and conjugated diene accumulation and the carcinogenicity of peroxisome proliferators.

The effects of long-term treatment with peroxisome proliferators on cell proliferation have been investigated. Marsman *et al*. (1988) compared replicative DNA synthesis in male F-344 rats receiving a diet containing 1.2% DEHP or 0.1% WY-14,643. A minipump technique allowed accurate quantification of low levels of replicative DNA synthesis in the rat liver. A large and equivalent increase in replicative DNA synthesis occurred in DEHP- and WY-14,643-fed rats during the first week of treatment, corresponding to chemically-induced liver growth. A sustained 5- to 10-fold increase in replicative DNA synthesis was found in rats receiving WY-14,643 from 39 to 365 days. Replicative DNA synthesis in DEHP-fed rats was at control levels at days 18, 39, 77 and 151, but increased to 70% above controls at 365 days. This study indicated a strong correlation between persistent cell proliferation and the high carcinogenicity of WY-16,643 as compared to DEHP. However, this picture is complicated by a recent study by Lalwani *et al*. (1985), which suggests that increases in replicative hepatic DNA synthesis caused by peroxisome proliferators may not be indicative of cell proliferation, but may rather indicate an increase in hepatic nuclear ploidy. This may indicate that prior studies which reported increases in cell proliferation using light microscopic evaluations of bromodeoxyuridine (BrdU)-labeled cell nuclei may have in fact been observing increases in hepatic nuclear ploidy. Additionally, some peroxisome proliferator-induced increases in hepatic cell number may be due to an inhibition of apoptosis (programmed cell death) (Roberts, 1996).

Peroxisome Proliferator Activated Receptor

Melnick *et al*. (1996) have reviewed the potential mechanisms of a number of nongenotoxic carcinogens including DEHP. The following extract on peroxisome proliferator activated receptor (PPAR) is taken from their paper:

> "The discovery of the peroxisome proliferator-activated receptor (PPAR) (Issemann & Green, 1990), a ligand-activated intracellular transcription factor, provides a mechanistic basis for understanding how peroxisome proliferators modulate gene expression leading to induction of peroxisomal enzymes. Ligand binding activates the receptor, which subsequently forms a heterodimer with the retinoid X receptor. It is this ternary complex which binds to specific DNA response elements, causing transcriptional activation of genes coding for peroxisomal enzymes (Kliewer *et al*., 1992; Issemann *et al*., 1993). Humans possess PPAR subtypes, including one that shows high homology with rodent PPAR-α and that can be activated by peroxisome proliferators (Sher *et al*.,1993). It is not known whether a peroxisome proliferator (or one of its metabolites) binds directly to the receptor or whether receptor activation is mediated by changes in cellular levels of an endogenous ligand (e.g., fatty acid or fatty acyl-CoA). Further research is needed on binding of exogenous and endogenous ligands to PPAR subtypes in rodent and human hepatocytes, dose-response comparisons of the transcriptional activation of peroxisomal genes in rodent and human hepatocytes, regulation of PPAR activity, and interindividual variability of PPAR in human populations.
>
> "**Effects in Humans**. The fact that hypolipidemia, one of the pleiotropic effects of peroxisome proliferators in rodents, is also induced by these drugs in humans demonstrates that humans are responsive to these chemicals. Moderate increases in peroxisome number or volume density have been observed in patients taking clofibrate or ciprofibrate (Ashby *et al*., 1994). Induction of peroxisomal proliferation in human hepatocyte cultures could not be demonstrated. This difference between *in vivo* and *in vitro* behavior may be related to culturing conditions, as insulin inhibits and dexamethasone stimulates fatty acid-induced transcription of PPAR and peroxisomal enzymes in rat hepatocytes both *in vivo* and *in*

vitro (Steineger *et al*., 1994). Effects of these factors in human hepatocytes need to be investigated."

Melnick *et al*. (1996) reviewed a number of nongenotoxic carcinogens including DEHP and concluded that: 1) many chemicals considered to be nongenotoxic carcinogens actually possess certain genotoxic activities, 2) some nongenotoxic activities may cause oxidative DNA damage and thereby initiate carcinogenesis, 3) cytotoxicity and mitogenesis do not reliably predict carcinogenesis and 4) a threshold tumor response is not an inevitable result of a receptor-mediated mechanism.

Gaylor and Zheng (1996) have also stated that: 1) a threshold dose is questionable if a nongenotoxic carcinogen acts via a cell receptor, 2) a nongenotoxic carcinogen that increases the cell proliferation rate via the cell division rate is not likely to have a threshold dose and 3) dose-response curves for cell proliferation and tumor incidence do not necessarily mimic each other.

Linear approach

According to the proposed draft guidelines for carcinogen risk assessment (U.S. EPA, 1996) the type of extrapolation employed for a given chemical depends on the existence of data supporting linearity or non-linearity or a biologically-based or case-specific model. When no data are available supporting either approach the default is to use a linear extrapolation. When data support both approaches and no model exists the default is to use both linear and non-linear methods. DEHP seems to fit this latter category with sufficient uncertainty about its mode of action to justify both approaches.

U.S. EPA has previously employed the linearized multistage model (LMS) with the datasets from the rodent cancer bioassays on DEHP conducted by the (NTP, 1982; Kluwe *et al*., 1982). Although four potencies or slope factors were determined for combined hepatocellular carcinoma and neoplastic nodules in male and female rats [0.0032 and 0.0045 $(mg/kg\text{-}day)^{-1}$, respectively], and for hepatocellular adenomas and carcinomas in male and female mice [0.014 and 0.010 $(mg/kg\text{-}day)^{-1}$, respectively], U.S. EPA selected the highest value of 0.014 $(mg/kg\text{-}day)^{-1}$] (IRIS, 1993). An alternative assessment by U.S. EPA's Office of Water (U.S. EPA, 1986b) employing revised human equivalent doses resulted in potencies of 0.00295, 0.00352, 0.00836 and 0.00473 $(mg/kg\text{-}day)^{-1}$, respectively. The highest value of 0.0084 $(mg/kg\text{-}day)^{-1}$ was selected by OEHHA as its oral potency value (DHS, 1988).

All of the potencies derived by U.S. EPA were scaled to human equivalent using $(body\ weight)^{2/3}$. The proposed 1996 guidelines for carcinogen risk assessment recommend a linear extrapolation approach based on the 95% lower bound on the dose which produces a 10% tumor incidence (LED_{10}) and inter-species scaling based on $(body\ weight)^{3/4}$. Table 3 summarizes the key rodent cancer bioassay datasets and the cancer potency values derived from them. In all cases the TOX_RISK (v.3.1, K.S. Crump Division, Clement International Corp., Ruston, LA) program was used to fit the multistage model to the quantal data sets. Inter-species scaling (rodent to human) was based on $(body\ weight)^{3/4}$ resulting in a potency correction factor of $(human\ body\ weight/\ animal\ body\ weight)^{1/4}$. The q_1* cancer potencies or the 95% upper-bound on the linear slope at low dose (LMS) were calculated directly by the program. To calculate cancer slope factors (CSFs) based on the LED_{10} or the 95% lower-bound on the dose that is predicted to give a 10% tumor incidence, the value predicted by the program in ppb diet units was first converted to mg/kg-day assuming 1.5 kg/day diet intake for a 70 kg human. The CSF is then calculated as $0.1/LED_{10}$ in units of $(mg/kg\text{-}day)^{-1}$. As can be seen from Table 4a the potency estimates for liver tumors are quite similar whether based on the q1* or the CSF ranging from a CSF of 9.8×10^{-4} for female rat

liver carcinoma and neoplastic nodules in the NTP (1982) study to a q_1* of 3.2×10^{-3} for male rat liver carcinoma and neoplastic nodules in the Corning Hazleton (1996) study. The best value is probably the CSF of 3.0×10^{-3} (mg/kg-day)$^{-1}$ for liver adenomas and carcinomas in the male rat from the same study. This can be compared with U.S. EPA's current value of 1.4×10^{-2} (mg/kg-day)$^{-1}$ or OEHHA's current oral value of 8.4×10^{-3} (mg/kg-day)$^{-1}$. For the CSF calculations we have employed a $p \geq 0.05$ criterion for the Chi-squared goodness of fit statistic.

Table 3a. Carcinogenic Potency Estimates for DEHP from Rodent Cancer Bioassays

Study / Data Set	**Doses (mg/kg-d)**	**Quantal Tumor Response**	**q_1* (mg/kg-d)$^{-1}$**	**χ^2**	**p**	**k**	**LED_{10} (mg/kg-d)$^{-1}$**	**CSF (mg/kg-d)$^{-1}$**
NTP (1982): 103 weeks treatment, 105 weeks duration								
M Rat, liver carcinoma or neoplastic nodule	0, 322, 674	3/50, 6/49, 12/49	1.9×10^{-3}	0.0	1.0	2	55.98	1.8×10^{-3}
F Rat, liver carcinoma or neoplastic nodule	0, 394, 774	0/50, 2/49, 8/50	1.0×10^{-3}	0.01	0.92	2	102.1	9.8×10^{-4}
M Mouse, liver carcinoma	0, 672, 1,325	9/50, 14/48, 19/50	2.6×10^{-3}	0.0017	0.97	2	40.16	2.5×10^{-3}
F Mouse, liver carcinoma	0, 799, 1,821	0/50, 7/50, 17/50	2.1×10^{-3}	0.0	1.0	2	50.76	2.0×10^{-3}
Hazleton (1996): 104 weeks duration.								
M Rat, liver carcinoma or neoplastic nodule	0, 5.8, 28.9, 146.6, 789.0	5/80, 5/50, 4/55, 11/65, 34/80	3.2×10^{-3}	0.86	0.84	4	33.41	3.0×10^{-3}
F Rat, liver carcinoma or neoplastic nodule	0, 7.3, 36.1, 181.7, 938.5	0/80, 4/50, 1/55, 3/65, 21/80	1.4×10^{-3}	7.92	0.02	4	73.16	1.4×10^{-3}

Note: The q_1* is the carcinogenic potency determined by the linearized multistage model. X^2 is the value of the chi squared goodness of fit statistic; p is the significance level of the chi squared value where a criterion of $p \geq 0.05$ is considered an adequate fit of the polynomial equation to the quantal tumor response data set; k is the number of non-zero doses used in the fitting procedure. The LED_{10} is the 95% lower confidence limit on the dose required to give a 10% tumor incidence. The CSF is the carcinogenic potency or cancer slope factor calculated from the LED_{10} (i.e., $0.1/LED_{10}$ = CSF).

Table 3b. Multistage Models fit to DEHP Rodent Cancer Bioassay Data

Study / Data Set	**Doses (mg/kg-day)**	**Quantal Tumor Response**	**Polynomial Coefficients**	
NTP (1982): 103 weeks treatment, 105 weeks duration.				
M Rat, liver carcinoma or neoplastic nodule	0, 322, 674	3/50, 6/49, 12/49	q_0=6.187540E-2 q_2=3.166839E-7	q_1=1.115209E-4
F Rat, liver carcinoma or neoplastic nodule	0, 394, 774	0/50, 2/49, 8/50	q_0=0.0 q_2=2.862112E-7	q_1=0.0
M Mouse, liver carcinoma	0, 672, 1,325	9/50, 14/48, 19/50	q_0=1.992340E-1 q_2=0.0	q_1=2.121160E-4
F Mouse, liver carcinoma	0, 799, 1,821	0/50, 7/50, 17/50	q_0=0.0 q_2=3.856678E-8	q_1=1.579497E-4
Hazleton (1996): 104 weeks duration				
M Rat, liver carcinoma or neoplastic nodule	0, 5.8, 28.9, 146.6, 789.0	5/80, 5/50, 4/55, 11/65, 34/80	q_0=7.560943E-2 q_2=0.0 q_4=0.0	q_1=6.176571E-4 q_3=0.0
F Rat, liver carcinoma or neoplastic nodule	0, 7.3, 36.1, 181.7, 938.5	0/80, 4/50, 1/55, 3/65, 21/80	q_0=2.598187E-2 q_2=0.0 q_4=2.198296E-13	q_1=1.150446E-4 q_3=0.0

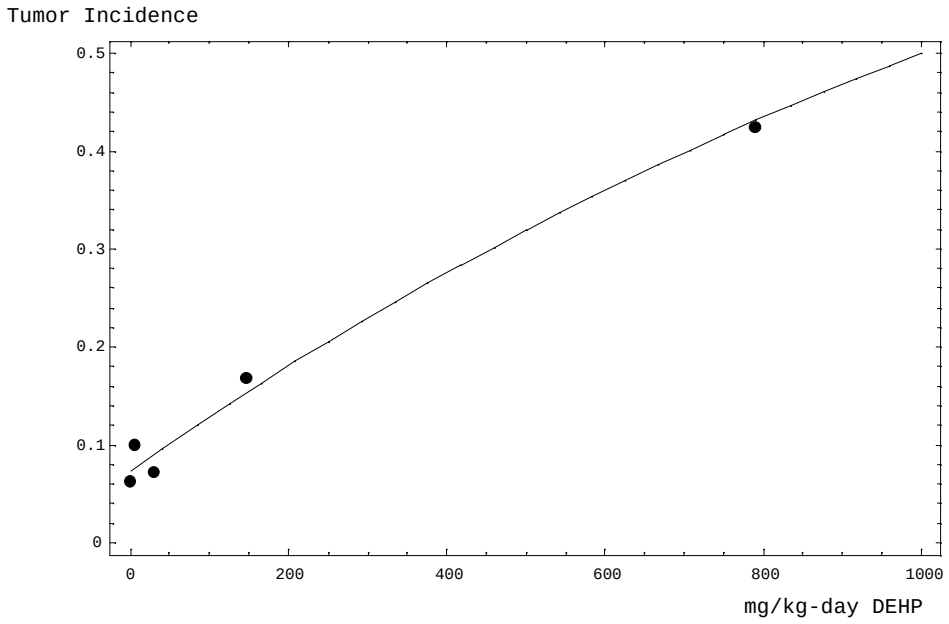


Figure 1. Multistage Fit of Hazleton '96 DEHP Male Rat Data

Non-linear approach

In the non-linear approach it is assumed that a practical threshold for carcinogenic effects exists at some dose below those employed in the cancer bioassays. The point of departure for this calculation would be an appropriate LED_{10} for tumor formation in the rodent bioassay (i.e., the 33.41 mg/kg-day value) for male rats in the Corning Hazleton study. This value can be treated as an LOAEL for DEHP-induced carcinogenicity in calculations of an adequate margin of exposure (MOE). Familiar uncertainty factors would be employed.

Noncarcinogenic Effects

The most sensitive noncarcinogenic endpoint is probably that for reproductive or developmental toxicity in the NTP (1984) study in mice. In this continuous breeding study an NOAEL of 0.01% DEHP in feed (14.2 mg/kg-day) was identified. Assuming 5.1 g/day of feed consumed and an average body weight of 36 g, the study doses were estimated as 0, 14.2, 141 and 425 mg/kg-day.

CALCULATION OF PHG

Carcinogenic Effects

Data from the NTP (1982) bioassay has been used by a number of agencies to calculate human carcinogenic risk estimates. Under the DHS carcinogen guidelines (DHS, 1985), DEHP is identified as a carcinogenic hazard because an oncogenicity bioassay conducted in male and female B6C3Fl mice and F-344 rats was considered to provide clear evidence of carcinogenicity for DEHP (NTP, 1982). Although this study has been criticized (Northrup *et al.*, 1982), U.S. EPA

(1986a) concluded that the evidence on potential carcinogenicity from animal studies is "sufficient," and that DEHP is a probable human carcinogen.

In 1986, U.S. EPA (1986a) summarized the weight-of-evidence regarding the oncogenic potential of DEHP:

> "DEHP administered in the diet at levels near the maximum tolerated dose (MTD) for 103 weeks has been shown to be carcinogenic in male and female F344 rats and B6C3Fl mice. The incidence of hepatocellular carcinoma or hepatic neoplastic nodules was significantly ($p < 0.05$) increased in male rats exposed at 12,000 mg/kg diet and female rats at 6,000 and 12,000 mg/kg diet. A significant ($p < 0.05$) dose-dependent trend of increasing liver neoplasms was reported for both sexes of rats. In mice treated at 3,000 and 6,000 mg/kg diet, hepatocellular carcinoma was significantly ($p < 0.05$) elevated in high-dose males and all treated females compared to controls. Both sexes showed a significant ($p < 0.05$) dose-dependent trend of increasing hepatocellular carcinoma.
>
> "No data regarding the carcinogenicity of DEHP to humans were available. No case reports or epidemiological studies on humans exposed to DEHP were located in the available literature. Thus, using the U. S. EPA (1984) Guidelines for Carcinogen Risk Assessment for evaluating the overall weight of evidence to humans, DEHP is most appropriately classified as a Group B2 chemical."

The data from the recent Corning Hazleton (1996) study essentially confirm the earlier NTP rat study with more dose levels. Due to the lack of clear indication of a threshold from the dose response data or any convincing mechanistic data or models, U.S. EPA's proposed cancer guidelines would suggest that a linear approach would probably be the most appropriate method to assess human cancer risks. In the calculations below both linear and non-linear approaches are presented.

Linear Approach

Assuming that the CSF of 3.0×10^{-3} $(mg/kg\text{-}day)^{-1}$ is the best estimate of cancer potency, a health-protective drinking water concentration (C) for DEHP (in mg/L) can be calculated using the general equation for carcinogenic endpoints:

$$C = \frac{BW \times R}{CSF \times L/day} = mg/L$$

where,

BW	=	Adult default male body weight (70 kg)
R	=	*De minimis* theoretical lifetime excess individual cancer risk level (10^{-6})
L/day	=	Volume of water consumed daily by an adult (2 L/day).

Therefore,

$$C = \frac{70 \text{ kg} \times 10^{-6}}{3 \times 10^{-3} \text{ (mg/kg-day)}^{-1} \times 2 \text{ L/day}}$$

$$= 0.0116 \text{ mg/L} = 0.012 \text{ mg/L (rounded)} = 12 \text{ ppb.}$$

A public health-protective concentration for DEHP in drinking water using the linear approach is 12 ppb.

Non-linear Approach

Assuming that a non-linear approach is more appropriate, a health-protective drinking water concentration (C, in mg/L) can be calculated following the general equation for carcinogenic endpoints:

$$C = \frac{LED_{10} \times BW \times RSC}{UF \times L/day}$$

where,

LED_{10}	=	95% Lower-bound on the dose that gives a 10% tumor incidence rate (use 33.4 mg/kg-day for DEHP)
BW	=	Adult default male body weight (70 kg)
RSC	=	Relative source contribution (use a default of 20% or 0.2 for DEHP)
UF	=	Uncertainty factors (use 1,000 for DEHP: 10-fold for the severity of the endpoint, 10-fold for human variability, and 10-fold for conversion of an LOAEL to an NOAEL)
L/day	=	Volume of drinking water consumed daily for an adult (2 L/day).

Therefore,

$$C = \frac{33.4 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.2}{1{,}000 \times 2 \text{ L/day}}$$

$$= 0.234 \text{ mg/L} = 0.23 \text{ mg/L (rounded)} = 230 \text{ ppb.}$$

Noncarcinogenic Effects

A health-protective drinking water concentration (C) for DEHP in drinking water (in mg/L) can be calculated following the general equation for noncarcinogenic endpoints:

$$C = \frac{NOAEL \times BW \times RSC}{UF \times L/day} = \text{mg/L}$$

where,

NOAEL	=	No-observed-adverse-effect-level (14.2 mg/kg-day)
BW	=	Adult default male body weight (70 kg)
RSC	=	Relative source contribution (default of 20% or 0.2 for DEHP)

UF = Uncertainty factor (use 1,000 for DEHP: 10-fold for inter-species extrapolation, 10-fold for human variability, and 10-fold for the developmental and reproductive endpoint)

L/day = Volume of drinking water consumed daily for an adult (2 L/day).

Therefore,

$$C = \frac{14.2 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.2}{1{,}000 \times 2.0 \text{ L/day}}$$

$$= 0.0994 \text{ mg/L} = 0.1 \text{ mg/L (rounded to nearest mg/L)} = 100 \text{ ppb.}$$

OEHHA concludes that the most sensitive endpoint for assessing potential human risk from chronic low level exposure to DEHP is likely to be the carcinogenic endpoint. Based on the lack of a clear dose-response indicative of a threshold for the carcinogenicity of DEHP demonstrated in rodent bioassays or other convincing mechanistic arguments as to the mode(s) of action of DEHP, current guidelines would propose a linear approach to assess human risks. From the calculation above, a public health-protective concentration based on a *de minimis* theoretical excess individual lifetime cancer risk level of 10^{-6} is 0.012 mg/L (12 ppb). For risk management purposes, public health-protective concentrations of 120 and 1,200 ppb can be calculated based on the linear approach at theoretical excess individual lifetime cancer risk levels of 10^{-5} and 10^{-4}, respectively. The value of 12 ppb based on the *de minimis* risk level is the lowest (i.e., most health-protective) of the calculated values above, both using the linear and non-linear approaches for cancer risk estimates and for the developmental and reproductive (noncarcinogenic) endpoint. Therefore, OEHHA calculates and adopts a PHG of 12 ppb for DEHP in drinking water which is justified based on the scientific analysis as well as being public health-protective.

RISK CHARACTERIZATION

DEHP is an artificial substance with no sources other than human activities. It is a widespread environmental contaminant, but levels in the general environment are generally low. Higher levels of exposure for some individuals potentially arise from substantial local concentrations in waste sites, industrial sites where DEHP is used or stored and from certain special situations such as the repeated use of medical devices containing DEHP-plasticized PVC plastics. Adverse health effects which may occur as a result of human exposure to DEHP include cancer, developmental toxicity and reproductive toxicity. While experimental data in rodents indicate a potential for synergistic interaction with other water contaminants, notably trichloroethylene, for selected reproductive toxicity endpoints, the relevance of these observations with respect to human exposures and toxicity is highly uncertain.

The carcinogenicity of DEHP was tested in two major series of experiments. In the first (NTP, 1982; Kluwe *et al*., 1982), both male and female rats exhibited significant dose-dependent increases in the incidence of liver neoplasms (hepatocellular carcinomas or hepatic neoplastic nodules). Similarly, both male and female mice exhibited significant dose-dependent increases in hepatocellular carcinomas. The survival and growth of animals in this study showed minimal impact in spite of the high doses of DEHP used. In the second series (Corning Hazleton, 1996), both rats and mice were studied but only the data from rats were available at the time of preparation of this report. Hepatocellular adenomas and carcinomas were increased significantly in both sexes at the highest dose level (12,500 ppm), and also a marginal increase of these tumors

was apparent in the next highest dose level (2,500 ppm) in males only. Increases in mononuclear cell leukemia were also noted in the high-dose groups. Effects of all types were minimal in the two lowest dose groups. In this series of experiments, both rats and mice were studied but full data were available only from the rats at the time of preparation of this report. Preliminary accounts of the mouse study indicate that the results are similar to the concurrent rat study, and to the NTP mouse study, with observation of hepatocellular carcinoma at the two highest dose levels. Both these series of studies appear to have been well designed and conducted, and there is little reason to doubt the conclusion that high doses of DEHP are carcinogenic to rodents.

A considerable number of studies in rodents (including the NTP long-term bioassay described in the section on carcinogenesis, and a separate NTP multi-generation reproductive toxicity study) have produced evidence that high doses of DEHP result in testicular atrophy, impaired male reproductive performance and various cellular and endocrine changes related to these findings. Several studies in mice and rats also indicate that gestational exposure to DEHP results in developmental toxicity, causing fetal death, reductions in neonatal weight and survival and various malformations. Postnatal exposure via milk also causes decreased weight gain, liver enlargement and peroxisome proliferation in the brain and liver, in exposed rat pups. The reproductive and developmental effects of DEHP have been primarily reported in rodents. Direct evidence of such toxic effects in humans does not exist, and systematic studies in non-rodent species are not available.

Many reports show that exposure of rodents to DEHP results in liver peroxisome proliferation. This response is visible microscopically as a massive increase in the number of peroxisomes (a small membranous organelle which contains various oxidative enzymes) in the hepatocytes, and also lesser increases in the number of mitochondria and lysosomes. These changes are associated with liver enlargement at the macroscopic level, and with various biochemical changes including increases in oxidative metabolism of fatty acids in both peroxisomes (by oxidase enzymes) and mitochondria (by β oxidation). Peroxisome proliferation is associated with various kinds of cell damage ascribed to excess of oxygen-containing reactive intermediates, including increases in lipid peroxidation, 8-hydroxyguanosine residues in DNA, and cell death. Increases in cell proliferation are typically noted within a few days of the start of exposure to DEHP, although this effect may be reduced or modified at later times during chronic exposures. A number of different chemicals besides DEHP have this effect on the rodent liver. Many of these compounds do not have obvious resemblance in chemical structure to DEHP, but belong to classes of chemical known to have hypolipidemic activity *in vivo*: some have been used as drugs to lower serum lipid levels (both in preclinical and clinical trials and as approved for human use).

Studies with DEHP and other peroxisome proliferation inducers in rodent, and in other mammalian species including primates, have shown that the rodent species show greater sensitivity to this effect than others. The highly visible histological changes and oxidation-related pathology are apparently not seen in non-rodent (including primate) species, although some of the biochemical alterations, including the hypolipidemic response to the drugs, have been seen in other species including humans. Some reports of increased peroxisome levels in humans exposed to large doses of DEHP or to hypolipidemic drugs have appeared, but in general such response are much less extreme and less sensitive than those characteristic of the rodent species. Nearly all of the reports of peroxisome proliferation in rodents relate to its occurrence in the liver. A few reports suggest that the response can occur in other tissues also but to a less extreme and visible degree; some of these suggest that the great difference in sensitivity of the rodent liver is not necessarily characteristic of extrahepatic tissues.

DEHP has generally been found to not induce gene mutations in *in vitro* and *in vivo* mutation assays. However, DEHP exposure may cause deleterious chromosomal effects such as chromosomal aberrations and aneuploidy. No DEHP-derived adducts have been found in DNA in exposed rodent liver, although 8-hydroxyguanosine residues considered characteristic of oxidative damage to DNA have been found.

The mechanism by which DEHP causes the carcinogenic effect observed in rodent liver is unknown, and may involve several different processes acting simultaneously. The hypothesis has been advanced that the carcinogenic effect is caused directly by the peroxisome proliferation, perhaps due to oxidative damage to DNA resulting in mutations of proto-oncogenes. Alternatively or additionally, the increases in cell proliferation seen after DEHP exposure may amplify either the effect of the background mutation rate, the enhanced mutation rate (if any) resulting from the oxidative DNA damage or any potential clastogenic effect. On the other hand there now appears to be sufficient evidence to conclude that although the carcinogenic response and the peroxisome proliferation response often appear in the same tissue and at similar dose levels the link is not necessarily so close as to support a strict causal association.

Recent studies have suggested that both the peroxisome proliferation response and the stimulus to cell proliferation (and perhaps also some other biochemical responses which are observed, such as induction of cytochrome P_{450} isoenzymes associated with Ω-1 hydroxylation of fatty acids) are independent responses to activation of an intracellular receptor ("peroxisome proliferation activation receptor," PPAR). It may be that activating this receptor results in sufficient perturbation of the normal control of cell division to explain, or at least contribute to, the observed carcinogenic and promoting activities of DEHP. Receptor interactions, either with PPAR or with steroid receptors, have been also considered probable causes of the developmental and reproductive effects. It does not appear likely that peroxisome proliferation is a sole or primary cause of these effects, although it may contribute to their severity especially at high doses and in some tissues.

This is an important difference, because the peroxisome proliferation response, at least in its extreme form with associated oxidative cell damage, appears to be specific to the rodent liver. On the other hand it seems that all mammalian species have a form of the PPAR, that it is widely distributed in extrahepatic tissues as well as the liver and that it is capable of responding to DEHP and other peroxisome proliferation inducing agents. If a receptor-based mechanism is in fact responsible for the carcinogenic, reproductive and developmental effects of DEHP then it is not possible to conclude with the information presently at hand that these effects would be seen only in rodents. However there may be modulating effects of the peroxisome response and also perhaps differences in pharmacokinetics which might result in greater sensitivity of rodents, compared to other species, to some or all of these effects.

The interpretation of the data on the biological mechanisms of DEHP toxicity have important implications for the selection of dose-response models for risk assessment. The classical "genotoxic" model for the carcinogenic effect, which has been generally assumed to mandate the assumption of low-dose linearity, may not be applicable to DEHP. The assumption of a simple oxygen-toxicity model based on peroxisome proliferation could be used to argue for a "true threshold" model, since presumably such effects would not be seen until a dose rate where the peroxisome induction and oxidant generation exceeded the reserve capacity of the detoxification and repair mechanisms. On the other hand a receptor model could show a number of possible dose-response shapes, ranging from Michaelis-Menten (which approximates to linearity at low

doses) to Hill equation or other cooperative binding relationships with various degrees of non-linearity between observed dose effect ranges and the environmental levels expected. On the whole, such mathematical constructions result in a an S-shaped or "hockey stick" type of curve rather than a true threshold. Although it may be possible to construct a receptor-response model which produces a true threshold, this certainly cannot be assumed. In addition, it has been shown that even where true thresholds apply to the dose response of an individual to a toxic effect, the existing background exposures (which may exceed the threshold for some exposed individuals) and/or variations in individual sensitivity may result in a population dose response which is linear (or at least close to linear) at low doses.

Dose-response assessments were performed for the two classes of hazard identified, carcinogenicity and developmental or reproductive toxicity. Because of the different nature of the hazards involved the risks of these different hazards are not necessarily directly comparable. However, as far as possible similar methodology and assumptions were used in both cases to facilitate a decision as to which hazard is likely to be limiting in determination of the risk estimates for these endpoints, based on animal studies, indicate that health risks would be minimal provided that daily intake does not exceed a level between 24 and 200 µg/day (depending on the endpoint, assumptions and methodology used in the analysis). These levels correspond to drinking water levels of 12 to 100 ppb, respectively. In view of this it is evident that use of OEHHA's calculated value of 12 ppb as a PHG provides adequate public health protection.

REFERENCES

Adams, W.J., Biddinger, G.R., Robillard, K.A., Gorsuch, J.W. (1995). A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. *Environ. Toxicol. Chem*. **14**(9): 1569-74.

Agarwal, D.K., Agarwal, S. and Seth, P.K. (1982). Effect of DEHP on drug metabolism, lipid peroxidation, and sulfhydryl content of rat liver. *Drug Metab. Dispos*. **10**:77-80.

Agarwal, D.K., Eustis, S., Lamb, J.C., Reel, J.R. and Kluwe, W.M. (1986). Effects of di(2-ethylhexyl)phthalate on the gonadal pathophysiology, sperm morphology, and reproductive performance of male rats. *Environ. Health Perspect*. **65**:343350.

ATSDR (1993). Toxicological profile for di-(2-ethylhexyl) phthalate (Update). TP-92/05. U.S.Department of Health and Human Services: Public Health Service, Agency for Toxic Substances and Disease Registry. Atlanta, GA, April 1993. 147 pp.

ATSDR (1987). Toxicological profile for di(2-ethylhexyl)phthalate. Prepared by Life Systems, Inc. for the Agency for Toxic Substances and Disease Registry. Published by Oak Ridge National Laboratory.

Albro, P.W. (1975). The metabolism of 2-ethylhexanol in rats. *Xenobiotica* **5**:625-636.

Albro, P.W. and Corbett, J.T. (1978). Distribution of di- and mono-(2-ethylhexyl) phthalate in human plasma. *Transfusion* **18**(6):750-5.

Albro, P. (1986). Absorption, metabolism, and excretion of DEHP by rats and mice. *Environ. Health Perspect*. **65**:293-298.

Albro, P.W. and Thomas, R.O. (1973). Enzymatic hydrolysis of di(2-ethylhexyl)phthalate by lipases. *Biochim. Biophys. Acta* **360:**380-390.

Albro, P.W. and Lavenhar, S. R. (1989). Metabolism of di(2-ethylhexyl)phthalate. *Drug Metab. Rev*. **21**(1):13-34.

Albro, P.W., Thomas R.O. and Fishbein, L. (1973). Metabolism of DEHP by rats: Isolation and characterization of the urinary metabolites. *J. Chromatog*. **76**:321-330.

Albro, P.W., Corbett, J.T., Schroeder, J.L., Jordan, S. and Matthews, H.B. (1982). Pharmacokinetics, interactions with macromolecules and species differences in metabolism of DEHP. *Environ. Health Perspect*. **45**:19-25.

Ashby, J., De Serres, F. J., Draper, M., *et al*. (1985). Evaluation of short-term tests for carcinogens. Report of the International Programme on Chemical Safety's collaborative study on *in vitro* assays. Progress in Mutation Research Vol. 5., Elsevier Science Publishers, New York, 752 pp.

Astill, B., Barber, E., Lington, A., Moran, E., Mulholland, A., Robinson, E., and Scheider, B. (1986). Chemical industry voluntary test program for phthalate esters: health effects studies. *Environ. Health Perspect.* **65**:329-336.

Autian, J. (1982). Antifertility effects and dominant lethal assays for mutagenic effects of DEHP. *Environ. Health Perspect.* **45**:115-118.

Background papers for workshop on DEHP (di[ethylhexyl]phthalate) risk assessment. April 19-20, 1988. Bethesda, MD.

Bell, F. (1976). Inhibition of hepatic sterol and squalene biosynthesis in rats fed DEHP. *Lipids* **11**:769-773.

Brain J.D., Blanchard J.D., Heyder J., Wolfthal S.F., Beck B.D. (1996). Relative toxicity of di(2-ethyl-hexyl) sebacate and related compounds in an *in vivo* hamster bioassay. *Inhalation Toxicol.* **8(6)**:579-93.

Bratt, H. and Batten, P. (1982). Imperial Chemical Industries PLC. As cited in U.S. CPSC, 1985.

Budroe J.D. and Williams G.M. (1993). Genotoxicity studies of peroxisome proliferators. Gibson GG, Lake B. *Peroxisomes: Biology and Importance in Toxicology and Medicine*. London: Taylor & Francis Ltd.: 525-68.

Butterworth, B.E., Bermudez, E., Smith-Oliver, T., Earle, L., Cattley, R., Martin, J., Popp, J.A., Strom, S., Jirtle, R-. and Michalopoulos, G. (1984). Lack of genotoxic activity of di(2-ethyl-hexyl)phthalate (DEHP) in rat and human hepatocytes. *Carcinogenesis* **5**:1329-1335.

Callahan, M.A., Slimak, M.W. and Gabel, N.W. (1979). Water-related environmental fate of 129 priority pollutants. U.S. Environmental Protection Agency, Washington, D.C. EPA-440/4-79-029b.

Calley, D., Autian, J. and Guess, W. (1966). Toxicology of a series of phthalate esters. *J. Pharm. Sci.* **55**:158.

Carpenter, C.P., Weil, C.S. and Smyth, H.F. (1953). Chronic oral toxicity of DEHP for rats, guinea pigs, and dogs. *Am. Med. Assoc. Arch. Ind. Hyg.* **8**:219-226.

Carter, J.E., Roll, D.B. and Peterson, R.V. (1974). The *in vitro* hydrolysis of di(2-ethylhexyl)-phthalate by rat tissues. *Drug Metab. Dispos.* **2**:341-344.

Chen, W.S., Kerkay, J., Pearson, K.H., Paganini, E.P. and Nakamoto, S. (1979). Tissue bis(2-ethylhexyl)phthalate levels in uremic subjects. *Anal. Lett.* **12**:1517-1535.

Chu, I., Dick, D., Bronaugh, R., and Tryphonas, L. (1996). Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. *Food Chem. Toxicol.* **34**(3):267-76.

Cole R.H., Frederick R.E., Healy R.. *et al*. (1984). Preliminary findings of the priority pollutant monitoring project of the nationwide urban runoff program. *J. Water Pollut. Control Fed.* **56**:898-908.

Conway, G.C. and Butterworth, B.E. (1988). Possible mechanisms in hepatocarcinogenesis by di(2-ethylhexyl)phthalate: implications for risk assessment. Cited in: Background Papers for Workshop on DEHP (Di[ethylhexyl]phthalate) Risk Assessment, Bethesda, Maryland. April 19-20, 1988.

Conway, J.G., Tomaszewski, K.E., Cattley, R.C., Marsman, D.S., Melnick, R.L. and Popp, J.A. (1988). Relationship of oxidative damage to carcinogenicity with the peroxisome proliferators di(2-ethylhexyl)phthalate and WY-14,643. *The Toxicologist* **5**:Abstract #644, 1988.

Corning Hazleton, Inc. (1996). Oncogenicity Study in Rats with Di(2-Ethylhexyl)phthalate Including Ancillary Hepatocellular Proliferation and Biochemical Analyses. Report No. CHV 633-134. Vol.1.

Daniel, J.W. and Bratt, H. (1974). The absorption, metabolism, and tissue distribution of di(2-ethylhexyl)phthalate in rats. *Toxicology* **2**:51-65.

Davis, B. J., Maronpot, R.R. and Heindel, J.J. (1994). Di-(2-ethylhexyl) phthalate suppresses estradiol and ovulation in cycling rats. *Toxicol. Appl. Pharmacol*. **128**: 216-223.

Deisinger, P.J., Barber, E.D., Schum, D.B. *et al*. (1991). A combined *in vitro*/*in vivo* model for estimating the dermal absorption of di(2-ethylhexyl)phthalate (DEHP) in man from contact with PVC film. *Toxicologist* **11**:95.

DHS (1988). Proposed Maximum Contaminant Level: Di(2-ethylhexyl) phthalate (DEHP). California Department of Health Services, Hazard Evaluation Section, Berkeley, California, currently Pesticide and Environmental Toxicology Section, Office of Environmental Health Hazard Assessment, Berkeley, CA.

Dirven, H.A.A.M., van den Broek, P.H.H., Arends, A.M.M., *et al*. (1993). Metabolites of the plasticizer di(2-ethylhexyl)phthalate in urine samples of workers in polyvinylchloride processing industries. *Int. Arch. Occup. Environ. Health* **64**(8):549-54.

Dostal, L.A., Weaver, R.P. and Schwetz, B.A. (1987a). Transfer of di(2-ethylhexyl) phthalate through rat milk and effects on milk composition and the mammary gland. *Toxicol. Appl. Pharmacol*. **91**: 315-325.

Dostal, L.A., Jenkins, W.L. and Schwetz, B.A. (1987b). Hepatic peroxisome proliferation and hypolipidemic effects of DEHP in neonatal and adult rats. *Toxicol. Appl. Pharmacol*. **87**:81-90.

Dostal, L.A., Chapin, R.E., Stefanski, S.A., Harris, M. W. and Schwetz, B.A. (1988). Testicular toxicity and reduced Sertoli cell numbers in neonatal rats by di(2-ethylhexyl) phthalate and the recovery of fertility as adults. *Toxicol. Appl. Pharmacol*. **95**: 104-121.

Douglas, R.G., Hugenholtz, A. P., and Blakley, D.H. (1986). Genetic toxicology of phthalate esters: mutagenic and other genotoxic effects. *Environ. Health Perspect.* **65**:255-262.

Egestad, B. and Sjöberg, P. (1992). Glucosidation as a new conjugation pathway for metabolites of bis(2-ethylhexyl) phthalate. *Drug Metab. Dispos. Biol. Fate Chem.* **20**(3):470-2.

Elliot, B.M. and Elcombe, C.R. (1987). Lack of DNA damage or lipid peroxidation measured *in vivo* in the rat liver following treatment with peroxisomal proliferators. *Carcinogenesis* **8**:1213-1218.

El Sisi, A.E., Carter, D.E. and Sipes, I.G. (1985). Dermal absorption and tissue distribution of phthalate esters. *The Toxicologist* **5**:246.

Eriksson, P. and Darnenid, P.O. (1985). Distribution and retention of some chlorinated hydrocarbons and a phthalate in the mouse brain during the pre-weaning period. *Toxicology* **37**:189-203.

Fratantoni J.C. (1992). Review: The platelet storage lesion: possible role of plasticizers? *Blood Cells* **18**:435-440.

Galloway, S.M., Bloom, A.D., Resnick, M., Margolin, B.H., Nakamura, F., Archer, P. and Zeiger, E. (1985). Development of a standard protocol for *in vitro* cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. *Environ. Mutagen.* **7**:1-51.

Ganning, A.E., Brunk, U., Edlund, C., Elhammer, A. and Dallner, G. (1987). Effects of prolonged administration of phthalate ester on the liver. *Environ. Health Perspect.* **73**:251-258.

Garvey, L.K., Swenberg, J.A., Hamm, T.E. and Popp, J.A. (1987). Di(2-ethylhexyl)phthalate: lack of initiating activity in the liver of female F-344 rats. *Carcinogenesis* **8**:285-290.

Gaunt, I.F., Butterworth, K.R. (1982). Autoradiographic study of orally administered di-(2-ethyl-hexyl)phthalate in the mouse. *Food Cosmet. Toxicol.* **20**:215-7.

Gennings, C. (1996). Economical designs for detecting and characterizing departure from additivity in mixtures of many chemicals. *Food Chem. Toxicol*. **34**:1053-1058.

Ghassemi, M., Quinlivan, S. and Bachmaier, J. (1984). Characteristics of leachates from hazardous waste landfills. *J. Environ. Sci. Health*. **19**:579-620.

Giam, C.S., Chan, H.S., Neff, G.S. and Atlas, E.L. (1978). Phthalate ester plasticizers: a new class of marine pollutant. *Science* **199**:419-421.

Glauert, H.P., Beer, D., Rao, M.S., Schwartz, M., Xu, Y., Goldsworthy, T.L., Coloma, J. and Pitot, H.C. (1986). Induction of altered hepatic foci in rats by the administration of hypolipidemic peroxisome proliferators alone or following a single dose of diethylnitrosamine. *Cancer Res.* **46**:4601-4606.

Gosselin, R.E., Smith, R.P. and Hodge, H.C. (1984). *Clinical Toxicology of Commercial Products*. Fifth Ed., Williams and Wilkins, Baltimore, MD., p. 204.

Gray, T.J. and Butterworth, K.R. (1980). Testicular atrophy produced by phthalate esters. *Arch. Toxicol.* (Suppl.) **4**:452-455.

Gray, T.J. and Gangolli, S.D. (1986). Aspects of the testicular toxicity of phthalate esters. *Environ. Health Perspect.* **65**:229-235.

Gray, T.J., Rowland, I.R., Foster, P.M. and Gangolli, S.D. (1982). Species differences in the testicular toxicity of phthalate esters. *Toxicol. Lett.* **11**:141-147.

Griffiths, W., Camara, P. and Lerner, K. (1985). Bis(2-ethylhexyl)phthalate, an ubiquitous environmental contaminant. *Ann. Clin. Lab. Sci.* **15(2)**:140-151.

Gupta, R.C., Goel, S.K., Earley, K., *et al*. (1985). ^{32}P- Postlabelling analysis of peroxisome proliferator-DNA adduct formation in rat liver *in vivo* and hepatocytes *in vitro*. *Carcinogenesis* **6**:933-936.

Harris, R.S., Hodge, H.C., Maynard, E.A. and Blanchat, H.J. (1956). Chronic oral toxicity of 2-ethylhexyl phthalate in rats and dogs. *Arch. Ind. Health* **13**:259-264.

Hawley, G. (1981). *The Condensed Chemical Dictionary*. Tenth Ed., Van Nostrand Reinhold Company, New York. p. 349.

Hillman, L.S., Goodwin, S.L. and Sherman, W.R. (1975). Identification and measurement of plasticizer in neonatal tissue after umbilical catheters and blood products. *N. Engl. J. Med.* **292**:381-386.

Hodge, H. (1943). Acute toxicity for rats and mice of 2-ethylhexanol and DEHP. *Proc. Soc. Exp. Med.* **53**:20-23.

Hornsby, P.J. and Harris, S.E. (1987). Oxidative damage to DNA and replicative lifespan in cultured adrenocortical cells. *Exp. Cell Res.* **168**:203-217.

Howe, R.B. and Crump, K.S. (1982). Global 82. A computer program to extrapolate quantal animal toxicity data to low doses. Prepared for the Office of Carcinogen Standards, OSHA, U.S. Department of Labor, under contract No. 41USC252C3.

Hrudey, S.E., Sergy, G.A. and Thackeray, T. (1976). Toxicity of oil sands, plant wastewaters, and associated organic contaminants. *Water Pollut. Res. Canada.* **76**:33-45.

Huber, W.W., Grasl-Kraupp, B. and Schulte-Hermann, R. (1996). Hepatocarcinogenic potential of di(2-ethylhexyl) phthalate in rodents and its implications on human risk. *Crit. Rev. Toxicol.* **26**(4):365-481.

IARC (1982). Di(2-ethylhexyl)phthalate. International Agency for Research on Cancer Monog. Eval. Carcinogenic Risk Chem. Humans 29:269-294. (also see errata in vol. 39, p455, 1983).

Ikeda, G.J., Sapienza, P.P., Couvillion JL, *et al*. (1980). Comparative distribution, excretion and metabolism of di-(2-ethylhexyl)phthalate in rats, dogs and miniature pigs. *Food Cosmet. Toxicol.* **18**:637-42.

IRIS (1993). Integrated Risk Information System. On-line File. U.S. Environmental Protection Agency, Washington, D.C.

Jäckh R, Rhodes C, Grasso P, Carter JT (1984). Genotoxicity studies on di-(2-ethylhexyl) phthalate and adipate and toxicity studies on di-(2-ethylhexyl)phthalate. *Food Chem Toxicol.* **22(2)**:151-5.

Jacobson, M.S., Kevy, S.V. and Grand, R.J. (1977). Effects of a plasticizer leached from polyvinylchloride on the subhuman primate: A consequence of chronic transfusion therapy. *J. Lab. Clin. Med*. **89**:1066-1079.

Kevy, S.V. and Jacobson, M.S. (1982). Hepatic effects of a phthalate ester plasticizer leached from polyvinylchloride blood bags following transfusion. *Environ. Health Perspect*. **45**:57.

Kihlstrom, I. (1983). Placental transfer of diethylhexyl phthalate in the guinea-pig placenta perfused in situ. *Acta Pharmacol. Toxicol. (Copen.)* **53**(1):23-7.

Klimisch H-J, Hellwig J, Kaufmann W, Jackh R (1991). Di-2-ethylhexyl Phthalate (DEHP). Investigation of Inhalation Toxicity in Rats After Repeated Exposure 28 D. *Human & Experimental Toxicology* **10**(1):68.

Klimisch HJ, Gamer AO, Hellwig J, Kaufmann W, Jackh R (1992). Di-(2-ethylhexyl) phthalate: a short-term repeated inhalation toxicity study including fertility assessment. *Food Chem. Toxicol.* **30**(11):915-9.

Kluwe, W.M., Haseman, J.K., Douglas, J.F. and Huff, J.E. (1982). The carcinogenicity of dietary di(2-ethylhexyl)phthalate (DEHP) in Fischer-344 rats and B6C3Fl mice. *J. Toxicol. Environ. Health* **10**(4-5):797-815.

Kluwe, W.M., Haseman, J.K. and Huff, J.E. (1983). The carcinogenicity of di(2-ethylhexyl)phthalate (DEHP) in perspective. *J. Toxicol. Environ. Health* **12**(l):159-169.

Kodeikh, N. (1985). Kinetic study of phthalate accumulation and elimination in a model of inhalational exposure in volunteers. *Probl. Khig.* **10**:81-7.

Kornbrust, D.J., Barfknecht, T.R. and Ingram, P.J. (1984). Effect of di(2-ethylhexyl)phthalate on DNA repair and lipid peroxidation in rat hepatocytes and on metabolic cooperation in Chinese hamster V-79 cells. *Toxicol. Environ. Health* **13**:99-116.

Krauskopf, L.G. (1973). Studies on the toxicity of phthalates via ingestion. *Environ. Health Persp.* **3**:61-72.

Lake, B.G., Gangolli, S.D., Grasso, P. and Lloyd, A.G. (1975). Studies on the hepatic effects of orally administered di(2-ethylhexyl)phthalate in the rat. *Toxicol. Appl. Pharmacol*. **32**:355-367.

Lake, B.G., Phillips, J.C., Linnell, J.C. and Gangolli, S.D. (1977). The *in vitro* hydrolysis of some phthalate diesters by hepatic and intestinal preparations from various species. *Toxicol. Appl. Pharmacol*. **39**:239-248.

Lake, B.G., Gray, T.J.B., Foster, J.R.,, Stubberfield, C.R. and Gangolli, S.D. (1984). Comparative studies on di(2-ethylhexyl)phthalate-induced hepatic peroxisome proliferation in the rat and hamster. *Toxicol. Appl. Pharmacol.* **72**:46-60.

Lake, B.G., Kozlen, S.L., Evans, J.G., Gray, T.J.B., Young, P.J. and Gangolli, S.D. (1987). Effect of prolonged administration of clofibric acid and di(2-ethylhexyl)phthalate on hepatic enzyme activities and lipid peroxidation in the rat. *Toxicology* **44**:213-228.

Lalwani ND, Reddy MK, Ghosh S, Barnard SD, Moletto JA, Reddy JK (1985). Induction of fatty acid β-oxidation and peroxisome proliferation in the liver of rhesus monkeys by DL-040, a new... *Biochem. Pharmacol.* **34**(19):3473-82.

Lamb, J.C. IV, Chapin, R.E., Teague, J., Lawton, A.D. and Reel, J.R. (1987). Reproductive effects of four phthalic acid esters in the mouse. *Toxicol. Appl. Pharmacol.* **88**: 255-269.

Lawrence, W., Malik, M., Turner, J., Singh, A. and Autian, J. (1975). A toxicological investigation of some acute, short-term and chronic effects of administering DEHP and other phthalate esters. *Environ. Res.* **9**:1-11.

Lhugenot, J.C., Mitchell, A.M., Milner, G., Lock, E.A. and Elcombe, C.R. (1985). The metabolism of DEHP and MEHP in rats: *in vivo* and *in vitro* dose and time dependency of metabolism. *Toxicol. Appl. Pharmacol.* **80**:11-22.

Lindgren, I., Linquist, N.G., Lyden, A., Olsson, T. and Ullberg, S. (1982). A whole body autoradiographic study on the distribution of ^{14}C-DEHP in mice. *Toxicology* **23**:149-158.

Liss, G.M., Albro, P.W., Hartle, R.W. and Stringer, W.T. (1985). Urine phthalate determinations as an index of occupational exposure to phthalic anhydride and DEHP. *Scand. J. Work Environ. Health* **11**:381-387.

Lutz, W.K. (1986). Investigation of the potential for binding of di(2-ethylhexyl)phthalate (DEHP) to rat liver DNA *in vivo*. *Environ. Health Perspect.* **65**:267-269.

Malcolm, A.R., and Mills, L.J. (1989). Inhibition of gap-junctional intercellular communication between Chinese hamster lung fibroblasts by di(2-ethylhexyl) phthalate (DEHP) and trisodium nitrilotriacetate monohydrate (NTA). *Cell Biol. Toxicol.* **5**:145-153.

Marcel, Y.L. (1973). Determination of di(2-ethylhexyl)phthalate in human blood plasma and cryoprecipitates. *Environ. Health Perspect.* **3**:119-121.

Marsman, D.S., Cattley, R.C. and Popp, J.A. (1987). Comparative effects of the peroxisomal proliferating agents, di(2-ethylhexyl)phthalate and WY-14,643 in rat liver. *The Toxicologist* **7**:Abstract #165.

Mathur, S. (1974). Phthalate esters in the environment: pollutants or natural products? *J. Environ. Quality* **3**:189.

Melnick, R.L., Morrissey, R.E., Tomaszewski, K.E. (1987). Studies by the National Toxicology Program on di(2-ethylhexyl)phthlate. *Toxicol. Ind. Health* **3**(1):99-118.

Melnick,R.L., Kohn, M.C. and Portier, C.J. (1996). Implications for risk assessment of suggested nongenotoxic mechanisms of chemical carcinogenesis. *Environ. Health Perspect*. **104**:123-134.

Merkle, J., Klimisch, H.J. and Jackh ,R. (1988). Developmental toxicity in rats after inhalation exposure of di-2-ethylhexylphthalate (DEHP). *Toxicol. Lett.* **42**(2):215-23.

Mikalsen, S.O. and Sanner, T. (1993). Intercellular communication in colonies of Syrian hamster embryo cells and the susceptibility for morphological transformation. *Carcinogenesis* **14**:251-257.

Milkoi, L.E., Aldyreva, M.V., Popova, T.B., Lopukhova, K.A., Mahavenko, Y.U., Malyar, L.M. and Shakhova, T.K. (1973). Health status of workers exposed to phthalate plasticizers in the manufacture of artificial leather and films based on PVC resins. *Environ. Health Perspect*. **3**:175-178.

Mitchell, F.E., Price, S.C., Hinton, R.H., Grasso, P. and Bridges, J.W. (1985). Time and dose-response study of the effects on rats of the plasticizer DEHP. *Toxicol. Appl. Pharmacol*. **81**:371-392.

Moody, D.E. and Reddy, J.K. (1978). Hepatic peroxisome (microbody) proliferation in rats fed plasticizers and related compounds *Toxicol. Appl. Pharmacol*. **45**:497-504.

Nakamura, Y., Yasuoki, Y., Tomita, I. and Tsuchikawa. (1979). Teratogenicity of DEHP in mice. *Toxicol. Lett*. **4**:113-117.

Narotsky, M.G., E.A.Weller, V.M.Chinchilli, and R.J.Kavlock. (1995). Nonadditive developmental toxicity in mixtures of trichloroethylene, di(2-ethylhexyl) phthalate, and heptachlor in a 5 x 5 x 5 design. *Fund. Appl. Toxicol*. **27**:203-216.

NAS (1977). *Drinking Water and Health*. Washington, D.C.: National Academy of Sciences. pp. 726-729.

NIH/EPA Chemical Information System (1981). Distribution Register of Organic Pollutants in Water (WDROP), Washington, D.C., C15 Project, Information Sciences Corporation.

Ng, K.M.E., Chu, I., Bronaugh, R.L., *et al*. (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl)phthalate: Comparison of *in vitro* and *in vivo* results in the hairless guinea pig. *Toxicol. Appl. Pharmacol.* **115**(2):216-23.

Nielsen, J., Akesson, B. and Skerfving, S. (1985). Phthalate ester exposure - air levels and health of workers processing polyvinylchloride. *Am. Ind. Hyg. Assoc. J.* **46**(11):643-647.

NOES. (1990). National Occupational Exposure Survey. National Institute of occupational Safety and Health, Cincinnati, OH.

Northrup, S., Martis, L., Ulbricht, R., Garber, J., Miripol, J. and Schmitz, T. (1982). Comment on the carcinogenic potential of di(2-ethylhexyl)phthalate. *J. Toxicol. Environ. Health* **10**:493-518.

NRC (1986). Toxicity of selected toxicants: di(2-ethylhexyl)-phthalate (DEHP) and mono(2-ethylhexyl)phthalate (MEHP). In: Drinking Water and Health. Vol. 6. National Research Council. National Academy Press, Washington, D.C. pp. 338-359 and 409-437.

NTP (1988). Reproduction and fertility evaluation of diethylhexyl phthalate (CAS No. 117-81-7) in CD-1 mice exposed during gestation. Final Report. National Toxicology Program PB88-204300. NTP88-092, Research Triangle Park, N.C.

NTP (1986). Reproduction and fertility evaluation of diethylhexyl phthalate (CAS No. 117-81-7) in Fischer 344 rats exposed during gestation. Final Report. National Toxicology Program PB87-119046. NTP86-309, Research Triangle Park, N.C.

NTP (1984). Diethylhexyl phthalate (DEHP): reproduction and fertility assessment in CD-1 mice when administered in the feed. Final Report. PB84-181734. National Toxicology Program NTP84-079, Research Triangle Park, N.C.

NTP (1982). Carcinogenesis bioassay of di(2-ethylhexyl)-phthalate (CAS No. 117-81-7) in F344 rats and B6C3F1 mice (feed study). National Toxicology Program NTP-80-37, NIH Publication 82-1773, Research Triangle Park, N.C.

Ochi, T. and Cerutti, P.A. (1987). Clastogenic action of hydroperoxy-5,8,11,13-icosatetraenoic acids on the mouse embryo fibroblasts C3H/l0T½. *Proc. Natl. Acad. Sci.* **84**:990-994.

Oishi, S. (1993). Strain differences in susceptibility to di-2-ethylhexyl phthalate-induced testicular atrophy in mice. *Toxicol. Lett.* **66**(1): 47-52.

Oishi, S. (1985). Reversibility of testicular atrophy induced by DEHP in rats. *Environ. Res.* **36**:160-169.

Oishi, S. and Hiraga, K. (1983). Testicular atrophy induced by DEHP: effect of zinc supplement. *Toxicol. Appl. Pharmacol.* **70**:43-48.

Parmar, D. Srivastava, S.P. and Seth, P.K. (1985). Hepatic mixed function oxidases and cytochrome P-450 contents in rat pups exposed to DEHP through mother's milk. *Drug Metab. Dispos.* **13**:368-370.

Patty, F. (1967). *Industrial Hygiene and Toxicology*. Vol. II. Interscience Publishers, New York, pp. 1904-1906.

Peakall, D.B. (1975). Phthalate esters: occurrence and biological effects. *Residue Rev.* **54**:1-41.

Pearson SD, Trissel LA. (1993). Leaching of diethylhexyl phthalate from polyvinyl chloride containers by selected drugs and formulation components. *Am. J. Hosp. Pharm.* **50**:1405-1409.

Peck, C.C., Albro, P.W., Hass, J.R., Odom, D.G., Barrett, B.B. and Bailey F.J. (1978). Metabolism and excretion of the plasticizer DEHP in man. *Clinical Res*. **26**:101A.

Perwak, J, Goyer, M, Schimke, G *et al. (*1981). An exposure and risk assessment for phthalic esters: di-(2-ethylhexyl) phthalate, di-n-butyl phthalate, dimethyl phthalate, di-n-octyl phthalate, butyl benzyl phthalate. EPA/440/4-81-020, NTIS No. PB85-211936. U.S. Environmental Protection Agency, Office of Water Regulations and Standards. Washington, D.C.

Pegg, D.G. (1979). Toxicity and biologic fate of DEHP following inhalation exposure in rats. Research Report No. 81-135. Unpublished study, August S. Cited in ATSDR, 1987.

Peterson, A., Draman, A., All, P. and Vinyard, D. (1974). Toxicity of plastic devices having contact with blood. NTIS Publication No. P3-233701. National Technical Information Service, U.S. Department of Commerce, Washington, D.C.

Phillips, B.J., James, T.E.B. and Gangolli, S.D. (1982). Genotoxicity studies of di(2-ethylhexyl)phthalate and its metabolites in CHO cells. *Mutat. Res*. **102**:297-304.

Phillips, B.J., Anderson, D. and Gangolli, S.D. (1986). Studies on the genetic effects of phthalic acid esters on cells in culture. *Environ. Health Perspect*. **65**:263-266.

Plonait SL, Nau H, Maier RF, Wittfoht W, Obladen M. (1993). Exposure of newborn infants to di-(2-ethylhexyl)-phthalate and 2-ethylhexanoic acid following exchange transfusion with polyvinylchloride catheters. *Transfusion* **33(**7):598-605.

Pollack, G.M., Buchannan, J.F., Slaughter, R.L., Kohli, R.K. and Shen, D.D. (1985a). Circulating concentrations of DEHP and its de-esterified phthalic acid products following plasticizer exposure in patients receiving hemodialysis. *Toxicol. Appl. Pharmacol*. **79**:257-267.

Pollack, G.M., Li, R.C., Ermer,, J.C. and Shen, D.D. (1985b). Effects of route of administration and repetitive dosing on the disposition kinetics of DEHP and its mono-de-esterified metabolite in rats. *Toxicol. Appl. Pharmacol.* **79**:246-256.

Popp, J.A., Garvey, L.K., Hamm, J.E. and Swenberg, J.A. (1985). Lack of hepatic promotional activity by the peroxisomal proliferating hepatocarcinogen di(2-ethylhexyl)phthalate. *Carcinogenesis* **6**:141-144.

Preat**,** V. and Roberfroid, M. (1987). Modulation of rat hepatocarcinogenesis by peroxisome proliferators. Second International Conference on Anticarcinogenesis and Radiation Protection, March 8-12, 1987. Gaithersburg, MD. Cited in: Conway, G.C. and Butterworth, B.E., 1988.

Rao, S.M. and Reddy, J.K. (1987). Peroxisome proliferation and hepatocarcinogenesis. *Carcinogenesis* **8**:631-636.

Rao, M.S., Usuda, N., Subbarao, V. and Reddy, J.K. (1987). Absence of gamma-glutamyltranspeptidase activity in neoplastic lesions induced in the liver of male F344 rats by di(2-ethyl-hexyl)phthalate, a peroxisome proliferator. *Carcinogenesis* **8**:1347-1350.

Reddy, J.K., Azarnoff, D.L. and Hignite, C.E. (1980). Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature* **283**:397-398.

Reddy, J.K., Reddy, M.K., Usman, M.I., Lalwani, N.D. and Rao, M.S. (1986). Comparison of hepatic peroxisome proliferative effect and its implication for hepatocarcinogenicity of phthalate esters, di(2-ethylhexyl)phthalate, and di(2-ethylhexyl)adipate with a hypolipidemic drug. *Environ. Health Perspect.* **64**:317-327.

Rhodes, C., Orton, T.C., Pratt, I.S., Batten, P.L., Bratt, H., Jackson, S.J., Elcombe, C.R. (1986). Comparative pharmacokinetics and subacute toxicity of di(2-ethylhexyl)phthalate (DEHP) in rats and marmosets: extrapolation of effects in rodents to man. *Environ. Health Perspect.* **65**:299-307.

Rhodes JE, Adams WJ, Biddinger GR, Robillard KA, Gorsuch JW (1995). Chronic toxicity of 14 phthalate esters to Daphnia magna and rainbow trout (Oncorhynchus mykiss). *Environ. Toxicol. Chem*. **14**(11):1967-76.

Roberts RA (1996). Non-genotoxic hepatocarcinogenesis: suppression of apoptosis by peroxisome proliferators. *Ann. NY Acad. Sci.* **27**:588-611.

Robinson, E., Lington, A., DiVincenzo, G., Chadwick, M., Branfman, A., Silveira, D. and McComosh, M. (1985). Non-linearity of metabolism of DEHP with dose and prior exposure. Paper presented at the Society of Toxicology Annual Meeting, March.

Roth, B., Herkenrath, P., Lehmann, H.J., *et al*. (1988). Di-(2-ethylhexyl)-phthalate as plasticizer in PVC respiratory tubing systems: indications of hazardous effects on pulmonary function in mechanically ventilated, preterm infants. *Eur. J. Pediatr.* **147**(1):41-6.

Rowland, I.R. (1974). Metabolism of di(2-ethylhexyl)phthalate by the contents of the alimentary tract of the rat. *Food Cosmet. Toxicol.* **12**:293-302.

Rubin, R.J. and Schiffer, C.A. (1976). Fate in humans of the plasticizer, di-2-ethylhexyl phthalate, arising from transfusion of platelets stored in vinyl plastic bags. *Transfusion* **16**(4):330-335.

Rubin RJ, Ness PM (1989). What price progress? An update on vinyl plastic blood bags. *Transfusion* **29**(4):358-361.

Sakurai, T., Miyazawa, S. and Hashimoto, T. (1978). Effects of DEHP administration on carbohydrate and fatty acid metabolism in rat liver. *J. Biochem*. **83**:313-320.

Salinas, J.A. (1988). Overview of risk assessment procedures used in six documents on di(2-ethylhexyl)phthalate. Cited in: Background Papers for Workshop on DEHP (Di[2-ethylhexyl]phthalate) Risk Assessment, Bethesda, MD. April 19-20, 1988.

Schmid, P. and Schlatter, C. (1985). Excretion and metabolism of DEHP in man. *Xenobiotica* **15**:251-256.

Schulz, C.O. and Rubin, R.J. (1973). Distribution, metabolism and excretion of DEHP in the rat. *Environ. Health Perspect.* **3**:123-129.

Scott, R.C., Dugard, P.H.,, Ramsey, J.D. and Rhodes, C. (1987). *In vitro* absorption of some o-phthalate diesters through human and rat skin. *Environ. Health Perspect.* **74**:223-227.

Seed, J.L. (1982). Mutagenic activity of phthalate esters in bacterial liquid suspension assays. *Environ. Health Perspect.* **45**:111-114.

Shaffer, C.B., Carpenter, C.P. and Smyth, H.F. (1945). Acute and subacute toxicity of DEHP with note upon its metabolism. *J. Indust. Hyg. Toxicol*. **27**:130-135.

Shiota, K. and Mima, S. (1985). Assessment of the teratogenicity of DEHP and MEHP in mice. *Arch. Toxicol*. **56**:263-266.

Shiota, K., Chou, M.J. and Nishimura, H. (1980). Embryo-toxic effects of DEHP and di-n-butyl phthalate in mice. *Environ. Res*. **22**:245-253.

Singh, A.R., Lawrence, W.H. and Autian, J. (1972). Teratogenicity of phthalate esters in rats. *J. Pharm. Sci*. **61**:51-55.

Singh, A.R., Lawrence, W.H. and Autian, J. (1974). Mutagenic and antifertility sensitivities of mice to di-(2-ethylhexyl)phthalate and di-methoxyethyl phthalate. *Toxicol. Appl. Pharmacol.* **29**:35-46.

Singh, A.R., Lawrence, W.H. and Autian, J. (1975). Maternal-fetal transfer of ^{14}C-di-2-ethylhexyl phthalate and ^{14}C-diethyl phthalate in rats. *J. Pharm. Sci*. **64**:1347-1350.

Sjoberg, P.O.J., Bondesson, U.G., Sedin, E.G. and Gustaffson, J.P. (1985a). Exposure of newborn infants to plasticizers: plasma levels of DEHP and MEHP during exchange transfusion. *Transfusion*. **25**:424-428.

Sjoberg, P., Bondesson, U. and Hammarlund, M. (1985b). Non-linearities in the pharmacokinetics of DEHP and metabolites in male rats. *Arch. Toxicol.* **58**:72-77.

Sjoberg, P., Bondesson, U., Kjellen, L., Lindquist, N.G., Montin, G. and Ploen, L. (1985c). Kinetics of DEHP in immature and mature rats and effect on testis. *Acta. Pharmacol. Toxicol*. **56**:30-37.

Sjoberg, P., Bondesson, U., Gray, T.J. and Ploen, L. (1986a). Effects of DEHP and five of its metabolites on rat testis *in vivo* and *in vitro*. *Arch. Toxicol.* **58**:72-77.

Sjoberg, P., Lindqvist, N.G. and Ploen, L. (1986b). Age-dependent response of the rat testes to di(2-ethylhexyl) phthalate. *Environ. Health Pers*pect. **65**: 237-242.

Tanaka, A., Adachi, T., Takahashi, T. and Yamaha, T. (1975). Biochemical studies on phthalic esters. I. Elimination, distribution and metabolism of di(2-ethylhexyl)phthalate in rats. *Toxicology* **4**:253-264.

Teirlynch, O.A. and Belpaire, F. (1985). Disposition of orally administered DEHP and MEHP in the rat. *Arch. Toxicol.* **57**:226-230.

Thomas, G.H. (1973). Quantitative determination and confirmation of identity of trace amounts of dialkyl phthalates in environmental samples. *Environ. Health Perspect.* **3**:23-28.

Thomas, J. and Thomas, M. (1984). Biological effects of di(2-ethylhexyl)phthalate and other phthalic acid esters. *CRC Crit. Rev. Toxicol.* **13**:282-317.

Thomas, J.A., Martis, L., Giovanetto, S., McCafferty, R.E. and Donovan, M.P. (1980). Biodistribution of monoethylhexyl phthalate (MEHP) in pregnant rabbits and neonatal rats. *Fed. Proc.* **39**:525.

Thomas, J.A., Schein, L.G., Gupta, P.K., McCafferty, R.E., Felice, P.R. and Donovan, M.P. (1979). Failure of monoethylhexyl phthalate to cause teratogenic effects in offspring of rabbits. *Toxicol. Appl. Pharmacol.* **51**:523-528.

Tomaszewski, K.E., Agarwal, D.K. and Melnick, R.L. (1986). *In vitro* steady-state levels of hydrogen peroxide after exposure of male F-344 rats and female B6C3Fl mice to hepatic peroxisome proliferators. *Carcinogenesis* **7**:1871-1877.

Tomita, I., Nakamura, Y., Aoki, N. and Inui, N. (1982). Mutagenic/carcinogenic potential of DEHP and MEHP. *Environ. Health Perspect.* **45**:119-125.

Tomita, I., Nakamura, Y., Yagi, Y. and Tutikawa, K. (1986). Fetotoxic effects of MEHP in mice. *Environ. Health Perspect.* **65**:249-254.

TRI (1987 to 94). Toxic Chemical Release Inventory, U.S. Environmental Protection Agency, database available through National Library of Medicine, Bethesda, MD.

Tsutsui, T., Watanabe, E., and Barrett, J.C. (1993). Ability of peroxisome proliferators to induce cell transformation, chromosome aberrations and peroxisome proliferation in cultured Syrian hamster embryo cells. *Carcinogenesis* **14**:611-618.

Turnbull, D. and Rodricks, J.V. (1985). Assessment of possible carcinogenic risk to humans resulting from exposure to di(2-ethylhexyl)phthalate (DEHP). *J. Am. Coll. Toxicol.* **4**:111-146.

Tyl, R.W., Price, C.J., Marr, M.C. and Kimmel, C.A. (1988). Developmental toxicity evaluation of dietary di(2-ethylhexyl)phthalate in Fischer 344 rats and CD-1 mice. *Fundam. Appl. Toxicol.* **10**:395-412.

U. S. CPSC (1985). Report to the U.S. Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on di(2-ethylhexyl)phthalate (DEHP). U. S. CPSC, Washington, D.C.

U.S. CPSC (1983). Briefing Package on DEHP in Children's Products to the Commissioners of the Consumer Product Safety Commission. section III - Dose-response Assessment, by Murray Cohn.

U.S. DHHS (1985). Public Health Service. Fourth annual report on carcinogens. Summary 1985. Washington, D.C.: U. S. Department of Health and Human Services. pp. 83-85.

U. S. EPA (1996). Proposed Guidelines for Carcinogen Risk Assessment. U.S. Environmental Protection Agency. *Federal Register* **61**(79):17960-18011. (April 23,1996).

U. S. EPA (1987a). Health Effects for selected phthalic esters. EPA/600/8-88/053, NTIS No. PB88-178934. U.S. Environmental Protection Agency, Environmental Criteria and Assessment Office. Cincinnati, OH.

U.S. EPA (1987b). Health and Environmental effects profile for phthalic acid alkyl, aryl and alkyl/aryl esters. Draft document dated September 1987. ECAO/OHEA, U.S. Environmental Protection Agency, Cincinnati, OH.

U. S. EPA (1986a). Evaluation of the potential carcinogenicity of bis(2-ethylhexyl)phthalate. Draft Report. Carcinogen Assessment Group, Office of Health and Environmental Assessment, U.S. Environmental Protection Agency. OHEA-C-073-45.

U. S. EPA (1986b). Drinking water criteria document for phthalic acid esters (PAEs). Draft document dated August 1986, with corrections as received March 7, 1988. ECAO/OHEA, U.S. Environmental Protection Agency, Cincinnati, OH.

U. S. EPA (1984). Proposed guidelines for carcinogen assessment, U.S. Environmental Protection Agency. 49 FR 46294-46307, November 23.

U. S. EPA (1983). Methods for chemical analysis of water and wastes. Office of Research and Development, Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency. Cincinnati, OH: EPA 600/4-79-020.

U. S. EPA (1980a). An exposure and risk assessment for phthalate esters. U.S. Environmental Protection Agency, Washington, D.C.

U. S. EPA (1980b). Ambient water quality criteria for phthalate esters. Office of Water Regulations and Standards. U.S. Environmental Protection Agency, EPA 440/5-80-067.

U. S. EPA (1980c). Hazardous waste management system: identification and listing of hazardous waste. U.S. Environmental Protection Agency, U.S. Code Fed. Regul., Title 40, Part 261;. Fed. Regist., 45(No. 138), pp. 47832-47834.

U. S. EPA (1978). Chemical hazard information profile - alkyl phthalates. Draft Report. U.S. Environmental Protection Agency, EPA 560/5-78-002.

Von Daniken, A., Lutz, W.K., Jackh, R. and Schlatter, C. (1984). Investigation of the potential for binding of di(2-ethylhexyl)phthalate (DEHP) and di(2-ethylhexyl)adipate (DEHA) to liver DNA *in vivo*. *Toxicol. Appl. Pharmacol.* **73**:373-387.

Walseth, F., Toftgard, R. and Nilsen, O.G. (1982). Phthalate esters. I. Effect on cytochrome P-450 mediated metabolism in rat liver and lung, serum enzymatic activities and serum protein levels. *Arch. Toxicol.* **50**:1-10.

Wams, T.J. (1987). Diethylhexylphthalate as an environmental contaminant - a review. *Sci. Total Environ.* **66**:1-16.

Ward, J.M., Rice, J.M., Creasia, D., Lynch, P. and Riggs, C. (1983) Dissimilar patterns of promotion by di(2-ethylhexyl)phthalate and phenobarbital of hepatocellular neoplasia initiated by diethylnitrosamine in B6C3Fl mice. *Carcinogenesis* **4**:1021-1029.

Ward, J.M., Ohshima, M., Lynch, P. and Riggs, C. (1984). Di(2-ethylhexyl)phthalate but not phenobarbital promotes N-nitrosodiethylamine-initiated hepatocellular proliferative lesions after short-term exposure in male $B6C3F_l$ mice. *Cancer Lett.* **24**:49-55.

Ward, J.M.,, Diwan, B.A., Ohshima, M., Hu, H., Schuller, H.M., and Rice, J.M. (1986). Tumor-initiating and promoting activities of DEHP *in vivo* and *in vitro*. *Environ. Health Perspect.* **65**:279-291.

Warf Institute (1976). Acute inhalation LC_{50} Sample LL-1132. Unpublished study. July 23. Cited in ATSDR, 1987.

Wilbourn, J. and Montesano, R. (1982). An overview of phthalate ester carcinogenicity testing results: The past. *Environ. Health Perspect.* **45**:127-128.

Wilkinson, C.F. (1997). Issues relating to potential human exposures of DEHP from use of intravenous medical devices. (Presentation Summary) Technology Sciences Group, Inc. Washington, D.C.

Williams, D.T. and Blanchfield, B.J. (1974). Retention, excretion and metabolism of DEHP administered orally to the rat. *Bull. Environ. Contam. Toxicol.* **11**:371-387.

Williams, G.M., Williams, H., Maruyama and Tanaka, T. (1987). Lack of initiating, promoting, or sequential syncarcinogenic effects of di(2-ethylhexyl)phthalate in rat liver carcinogenesis. *Carcinogenesis* **8**:875-880.

Williams M D, Adams W J, Parkerton T F, Biddinger G R, Robillard, K A (1995). Sediment sorption coefficient measurements for four phthalate esters: Experimental results and model theory. *Environ. Toxicol. Chem.* **14**(9):1477-1486.

WHO (1992). *Environmental Health Criteria* **131**: Diethylhexyl Phthalate. World Health Organization, Geneva, Switzerland. 141pp.

Yagi, Y., Nakamura, Y., Tomita, I., Tsuchikawa, K. and Shimoi, N. (1980). Teratogenic potential of di- and mono-(2-ethylhexyl)phthalate in mice. *J. Environ. Pathol. Toxicol.* **4**:533-539.